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RELATIVE SUSCEPTIBILITY TO CITRUS-CANKER OF DIFFERENT SPECIES AND HYBRIDS OF THE GENUS CITRUS, INCLUDING THE WILD RELATIVES [1]

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INTRODUCTION

In a preliminary report (*6*)[3] the senior author briefly described the results obtained under greenhouse conditions for a period of six months on the susceptibility and resistance to citrus-canker of a number of plants including some of the wild relatives, Citrus fruits, and hybrids of the genus Citrus. Since that time the plants reported on have been under close observation; a third experiment has been started, and many inoculations have been made in the isolation field in southern Alabama during the summers of 1917, 1918, and 1919. Many more plants have been successfully inoculated; others have proved to be extremely susceptible; while some of those tested still show considerable resistance. The results obtained up to November 1, 1919, are described in this report.

EXPERIMENTAL METHODS

In the greenhouse, the methods used and the conditions governing the inoculations described in the preliminary report were closely followed. The same strain of the organism was used and was applied in the

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[3] Reference is made by number (italic) to "Literature cited," pp. 361–362.

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same manner—that is, infusions of 48-hour-old cultures of *Pseudomonas citri* Hasse in beef bouillon were sprayed on the foliage of the plants by means of an atomizer. In no cases were punctures made, but wounds and scratches were present on some of the leaves. The plants all received identical treatments and were under approximately the same conditions.

The plants in experiment I were inoculated August 27 and September 12, 1917, and those in experiment II were inoculated October 23 and November 9. On March 23, 1918, the plants were all trimmed or cut back to force new growth, and some new numbers were placed in the screen cases together with the others. All of these were then inoculated in the usual manner. On the same date, the plants in experiment III were inoculated and have remained in the glass cases. Thus, after initial infection was obtained, natural inoculations of the remainder of the plants were counted on entirely. Therefore, natural infection took place on the greater part of the plants reported on in the following pages, especially on the more resistant plants.

In the isolation field, plantings were made in 1917, 1918, and 1919. Some of the nonhardy relatives and Citrus fruits were killed by hard winters of 1917–18 and 1918–19, but the majority of the plants survived. Inoculations in the field were started in September, 1917, by Mr. D. C. Neal and were later continued by Mr. J. Matz up to November 1, 1918, when the plants were banked for the winter. During the 1919 season the inoculations and observations in the field were made by the junior author.[1] Some of the plants were inoculated only once or twice, while others were sprayed with the inoculum regularly every week throughout the season. The time chosen for inoculation varied, but as a rule the late afternoon was chosen. A large number of natural infections took place after canker had been established on some of the plants.

The seasons of 1917 and 1918 were normal so far as climatic conditions were concerned. However, during the 1919 season the temperature was high, and together with an excessive and frequent rainfall it afforded not only ideal conditions for plant growth but also for the most rapid infection and development of canker.

Unless otherwise stated, all the plants reported on were in a good growing condition, and the organism was reisolated from the doubtful canker spots, especially in the case of the wild relatives. It must also be borne in mind that the plants used were for the most part small seedlings or nursery stock. Thus, the size of the plants and the conditions under which the inoculations were carried out made them more susceptible to canker. Plants reported here as susceptible would probably show more resistance under orchard conditions. No doubt maximum susceptibility was obtained with the plants experimented upon.

[1] The senior author is solely responsible for all conclusions drawn from the results of the three years' work.

SUSCEPTIBILITY OF NONRUTACEOUS PLANTS

Melia azedarach L., China berry, in field, 1919.

Inoculations were attempted on this plant in the field for the reason that it is the native host of the Citrus white fly. Needle prick and spray inoculations in the field under the most favorable conditions for the development of canker were negative.

Lee and Merrill (5) have reported successful inoculations of the stem and petioles of *Lansium domesticum* with *Pseudomonas citri*. This plant belongs to the same family as the China berry.

SUSCEPTIBILITY OF WILD RELATIVES OF THE GENUS CITRUS

RUTACEOUS PLANTS NOT CLOSELY RELATED TO THE GENUS CITRUS

Xanthoxylum sp. (CPB 11269, seedling), III.[1]

So far no canker spots have been found on the plants.

Casimiroa edulis Lav. and Lex. White sapote (CPB 7923, seedlings), I, II,[2] in field, 1919.[3]

At the October, 1918, readings a few nontypical spots were observed on several of the young leaves of the plant in experiment I. They occurred only at the wounds and scratches, no spots being found on the unbroken surface of the leaves. New spots have appeared from time to time, but in all cases they have occurred at wounds and remained unruptured. The spots (Pl. 57) are small (about 0.5 mm. in diameter), light colored, slightly raised, compact, and unruptured. They do not have an oily outline. No yellow zone is present. No positive infections were obtained in the field.

As there are three varieties of the white sapote being grown in California and Florida for its fruit, it is of interest to note that it can be successfully inoculated under greenhouse conditions, although it does show considerable resistance to citrus-canker.

Glycosmis pentaphylla DC. (CPB 2905, seedlings), II, III,[2] in field, 1918.

This is one of the few relatives tested which has, so far, remained immune to canker in both the greenhouse and field.

Claucena lansium Skeels. Wampi (CPB 7936, seedlings), I, II,[2] in field, 1917 and 1918.

A few small, nontypical, oily spots appeared on the leaves of the plants in both experiments. The spots are typical of those found on the wild relatives. Repeated inoculations in the field gave negative results.

Chalcas exotica Millsp. (*Murraea exotica* L.). Orange jessamine (CPB 7975A, seedlings), I, II, in field, 1917 and 1918.

During July, 1918, a few nontypical spots were observed on the young leaves of the plants in experiment II.

The spots resemble those on *Casimiroa edulis* in general appearance, except that they are somewhat larger and of a more oily character. A few new spots have developed since that time. The plants are only very weakly positive, and the period of incubation is rather long. The spots in all cases were at wounds and unruptured. In no case were positive results obtained in the field in spite of repeated inoculations.

RUTACEOUS PLANTS BELONGING TO TRIBE CITREAE

Subtribe Aeglinae (Hardshell Fruits)

Aegle marmelos Correa. Bael fruit (CPB 7983, seedlings), I, II, in field, 1918.

During the summer months these plants made a splendid growth and produced an abundance of new foliage. At the July, 1918, reading several small spots typical of

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[1] Roman numerals refer to the number of the inoculation experiment in the greenhouse.

[2] Included in experiments of March 21, 1918.

[3] Date of planting in the isolation field.

those found on *Casimiroa edulis* were observed, occurring at wounds and scratches. Spots have appeared occasionally since that time, but in every case they were observed along scratches and wounds and remained unruptured. All inoculations in the field were negative even at wounds.

Aeglopsis Chevalieri Swingle. (CPB 7633 and 7772, seedlings and cuttings), II and I,[1] in field, 1918.

The plants, although producing an abundance of new growth, have remained free from canker, in both the greenhouse and field.

Chaetospermum glutinosum (Blanco) Swingle (*Limonia glutinosa*, Blanco). Tabog (CPB 7799 and 7814, seedlings), I, II and I,[1] II,[1] III, in field, 1917 and 1918.

An abundance of new foliage was produced by all the plants, and thus they have been in an excellent condition for infection. All five of the plants have developed canker. The spots first appeared in April, 1918, three weeks after the last inoculation. The spots were at first small and nontypical (Pl. 58 A), but as they increased in numbers they became more and more typical. At the last reading the percentage of infected leaves ranged from 10 to 30, and from a few small, oily, unruptured spots (Pl. 58, B) to many medium-sized, ruptured spots (Pl. 58, C). No spots have been found on the twigs.

The small, unruptured spots generally appeared at wounds or scratches and resemble those described for *Casimiroa edulis*. The more normal spots are medium-sized, of a brick color, almost flat, compact, and slightly corky. They do not break through the upper surface but appear as a flat, discolored spot. The oily outline is very indistinct around the unbroken spots, and the yellow zone is absent. Vigorous colonies of *Pseudomonas citri* were isolated from these ruptured spots.

Unfortunately these plants are very susceptible to low temperatures and have been killed in the field each season, so that no thorough test of their susceptibility has been made. However, judging from the susceptibility shown in the greenhouse, they should be successfully inoculated in the field under favorable conditions. No positive results have been obtained so far, although the plants were repeatedly inoculated during the summers of 1917 and 1918.

Balsamocitrus Dawei Stapf. (CPB 2920, on *Aeglopsis Chevalieri*), III, in field, 1917 and 1918.

This is a large tree found in the forests of east central Africa at an altitude of 2,000 to 3,000 feet. The plant, although making a rapid growth, has remained free from canker in the field and the greenhouse.

Subtribe Feroninae (Hardshell Fruits)

Feronia limonia (Corr.) Swingle (*F. elephantum* Corr.). Wood-apple (CPB 2763, seedlings), I, II, in field, 1917 and 1918.

A few oily, unruptured spots were observed at the July, 1918, reading. The spots have become more numerous and are scattered over the new foliage, especially at wounds, but remain small and unruptured. They are typical in all respects to those described for *Casimiroa edulis*. No positive results have been obtained in the field.

Feroniella lucida Swingle. Kavista Batu (CPB 7882, seedlings), I, II, in field, 1917 and 1918.

Some small, very slow-growing, oily spots are scattered over the new foliage. They resemble in all respects those found on *Feronia limonia*. Repeated inoculations in the field have been negative.

[1] Included in experiments of March 21, 1918.

SUBTRIBE LAVANGINAE

Hesperthusa crenulata Roem. Naibel (CPB 2759, seedlings), II, III, in field, 1917 and 1918.

Because the spots produced were so nontypical the susceptibility of these plants was doubted until cankers developed on the twigs and branches. The spots (Pl. 59, B) are small and nontypical, although 90 per cent of the new leaves on the plants are infected. On the twigs they are rather numerous, flat, very oily, and apparently ruptured. In the field the few spots formed on the twigs have remained unruptured, very oily, and slightly raised. On the leaves the spots are nontypical, few, and in some cases slightly ruptured.

Triphasia trifolia P. Wilson. Lime berry (CPB 2689A and 7780, seedlings), I, II, and II, in field, 1918.

The plants have remained free from canker in both the field and greenhouse.

Severinia buxifolia Ten. (CPB 2760, cuttings and seedlings), I, II, in field, 1917, 1918, and 1919.

Like Triphasia, the plants are apparently immune.

SUBTRIBE CITRINAE

Citropsis Schweinfurthii Swingle and M. Kellerman. African cherry orange (CPB 11260, seedlings), I,[1] II, in field, 1917 and 1918.

Several small spots have developed along a wound on one leaf, while several small, scattering, and unruptured spots were found on a few young leaves. The spots are typical of those found on *Casimiroa edulis*. No positive results have been obtained in the field.

Atalantia citrioides Pierre. (CPB 7534, cuttings), I, II (2 plants), III, in field, 1918.

Canker spots first appeared on the plants in experiment I in May, 1918. Since that time all plants have become infected, the spots being well distributed over the new foliage.

The spots (Pl. 59, A) are small to medium, of a brick color, round, flat, and sometimes breaking out in a corky mass. Only a slight depression is visible on the upper surface. The oily outline is very distinct, and no yellow margin is present. The spots are somewhat similar to those described for Chaetospermum, and the plants are almost as susceptible. During 1918 no positive results were obtained on the few plants in the field.

Atalantia ceylonica Oliver (*Rissoa ceylonica*, Arn.). (CPB 11225, seedling), III.

A few oily spots (Pl. 59, A, center) have been produced on all the leaves of the plant. They are present also on the twigs. The spots are identical with those described on *A. citrioides*, although the plant is slightly more susceptible.

Poncirus trifoliata (L.) Raf. (*Citrus trifoliata* L.). Trifoliate orange (Seedlings, Alabama), I, II, III, in field, 1917.

All the plants (Pl. 65, A) included in the experiments have proved to be extremely susceptible in both the field and the greenhouse. In experiment III the plant was killed outright by the heavy canker infection. Leaves, thorns, twigs, branches, and even the old wood were attacked. As a rule, all the spots on the leaves are small to medium sized and very numerous, while on the stem they are large, girdling, and corky.

Poncirus trifoliata is extremely susceptible and therefore will always be a menace to complete eradication of canker in Alabama, especially since it has been found

[1] Included in experiments of March 21, 1918.

that canker may lie dormant in the bark tissues of the old wood and overwinter for a period of six months (7).

Eremocitrus glauca (Lindl.) Swingle (*Triphasia glauca* Lindl.; *Atalantia glauca* Benth.). Australian desert kumquat (CPB 7239 and 7397, seedlings), I, II, and III, in field, 1917.

All plants have shown infection, varying with their condition. Canker (Pl. 59, D) has been observed on the leaves, thorns, twigs, and old wood. A considerable degree of susceptibility is shown; and, under favorable conditions, the species should be successfully inoculated in the field, although such attempts have proved negative so far.

Fortunella margarita (Lour.) Swingle (*Citrus margarita* Lour.). Oval kumquat (CPB 7597, seedlings), I, II, III, in field, 1918.

Fortunella crassifolia Swingle. Meiwa kumquat (CPB 11047, seedlings), I, II (2 plants), III, in field, 1917 and 1918.

Fortunella japonica (Thunb.) Swingle (*Citrus japonica* Thunb.). Round kumquat (CPB 11301, seedlings), I, III, in field, 1918.

Fortunella Hindsii (Oliver) Swingle (*Sclerostylis Hindsii* Champ., *Atalantia Hindsii* Oliver). Hongkong wild kumquat (CPB 11046C and 11046A, seedlings), I, II, III, and I,[1] in field, 1917 and 1918.

All four species of kumquats have been successfully inoculated, although in all cases with some difficulty. From the results so far obtained no one of the first three species appears to be more susceptible than the other, the amount of infection depending on the growing condition of the individual. Judging from the type and number of spots (Pl. 60, C) *Fortunella Hindsii* is the most susceptible in that the spots are ruptured and corky.

As a rule, the spots on the other three species are characterized by being small, slow-growing, scattering, very dark, compact, and unruptured (Pl. 60, A). A few slightly ruptured, corky spots have been found on the plants, but usually at wounds.

Three plants of *Fortunella Hindsii* have been successfully inoculated in the field. A few minute infections were obtained on *Fortunella margarita* and *Fortunella japonica* during August, 1919. Plants of the oval kumquat, budded on *Poncirus trifoliata*, were inoculated in the field every week during the growing season of 1918 with negative results.

Microcitrus australasica (Muell.) Swingle (*Citrus australasica*, Muell.). Finger lime (CPB 7600 and 7600B, cuttings and seedlings), I, II, III, and II, in field, 1917.

Microcitrus australasica var. **sanguinea** Swingle. (CPB 7775B, cutting), II.

Microcitrus Garrowayi (Bail.) Swingle (*Citrus Garrowayi*, Bail.). Garroway's finger lime (CPB 11008, cuttings), I, II, III.

Microcitrus australis (Planch.) Swingle (*Citrus australis*, Planch.). Dooja (CPB 7307 and 7427, cuttings and seedlings), I, II, in field, 1917.

The last two species have proved to be quite easily infected with canker, but it was not until quite recently that *Microcitrus australasica* and its variety *sanguinea* became infected. Here infection is limited to a few scattering, small, slow-growing, dark, oily spots with an occasional spot on the thorns and twigs. On *M. australis* and *M. Garrowayi* from 30 to 90 per cent of the leaves have tiny, scattering, compact spots (Pl. 59, C), which do not penetrate through the leaf. Thorn, twig, and stem infections are also severe, the spots being ruptured and of a girdling type, resembling

[1] Included in experiments of March 21, 1918.

somewhat the loose, corky spots on *Poncirus trifoliata*. During the 1919 season *M. australis* was severely infected in the field, leaves, twigs, and branches being attacked. This species has shown almost as much susceptibility as *P. trifoliata*. Some leaf and stem cankers have also developed on *M. australasica*. However, it is much more resistant than *M. australis*.

In Table I the data on the susceptibility of the wild relatives of Citrus obtained by Lee (4) are listed for comparison with those reported on by the senior author. Lee worked in the open under field conditions at the Lamao Experiment Station, P. I., while the senior author, using the same type of plants, carried on his inoculation experiments in the greenhouses at Auburn, Ala. None of the field results are included in the table, since with few exceptions they were of a negative nature.

TABLE I.—*Findings of Lee and Peltier on the susceptibility of the Citrus relatives to citrus-canker*

Genus and species.	Lee's results.	Peltier's results.	Remarks.
RUTACEOUS PLANTS NOT CLOSELY RELATED TO THE GENUS CITRUS.			
Xanthoxylum rhetsa	Negative	Not tested	Immune.
Xanthoxylum sp.	Not tested	Negative	Do.
Evodia ridleyei	Positive	Not tested	Leaves and stem positive.
Evodia latifolia	do	do	Stem only.
Melicope triphylla	do	do	Do.
Casimiroa edulis	Not tested	Positive	Leaves only.
Toddalia asiatica	Positive	Not tested	Leaves and stem positive.
Glycosmis pentaphylla	Not tested	Negative	Immune.
Chalcas exotica	Positive	Positive	Lee, petioles and stem weakly positive; Peltier, leaves weakly positive
Clausena lansium	do	do	Do.
TRIBE CITREAE. Subtribe Aeglinae.			
Aegle marmelos	Negative	Positive	Leaves only.
Aeglopsis Chevalieri	Not tested	Negative	
Chaetospermum glutinosum	Positive	Positive	Susceptible.
Balsamocitrus gabonensis	Negative	Not tested	Immune.
Balsamocitrus Dawei	Not tested	Negative	Do
Subtribe Feroniinae.			
Feronia limonia	Positive	Positive	Lee, leaves and stem positive; Peltier, leaves only.
Feroniella lucida	do	do	Lee, stems only positive; Peltier, leaves only.
Subtribe Lavanginae.			
Hesperethusa crenulata	Positive	Positive	Susceptible.
Triphasia trifolia	Negative	Negative	Immune.
Paramignya longipedunculata.	Positive	Not tested	Leaves and stems positive.
Severinia buxifolia	Negative	Negative	Immune
Subtribe Citrinae.			
Citropsis Schweinfurthii	Positive	Positive	Lee, leaves and stems positive; Peltier, leaves only positive.
Atlantia citrioides	do	do	Lee, leaves and stems positive; Peltier, leaves very easily infected.
Atalantia ceylonica	Not tested	do	Leaves and stems easily infected
Atalantia disticha	Positive	Not tested	Leaves and stems only weakly positive.
Poncirus trifoliata	Not tested	Positive	Extremely susceptible.
Eremocitrus glauca	Positive	do	Susceptible.
Fortunella margarita	Not tested	do	Resistant.
Fortunella japonica	Positive	do	Do.
Fortunella crassifolia	Not tested	do	Do.
Fortunella Hindsii	Positive	do	Susceptible.
Microcitrus australasica	do	do	Somewhat susceptible.
Microcitrus var. *sanguinea*	Not tested	do	Do.
Microcitrus Garrowayi	do	do	Susceptible.
Microcitrus australis	Positive	do	Do.

Not only the conditions governing the inoculations but even the methods used were widely different in the two experiments. Lee (*4*) describes his method of inoculating as follows:

> In making the inoculations an infusion of the organism was painted upon the leaf blade, midrib, petiole, or stem, as the case might be, with a small camel's-hair brush, and then the tissue was punctured through the coating of infusion with a needle. The inoculated twig was maintained in a moist condition by wrapping it in paraffin paper, including with the twig also a small piece of moistened cotton.

The senior author of the present paper, on the other hand, used infusions of the canker organism, which were sprayed directly on the plants in the screened cases by means of an atomizer. In no case were punctures resorted to, although wounds and scratches were present at all times on some of the leaves. It should also be noted that natural infections took place from the more susceptible plants to the majority of the wild relatives. (See dates of inoculations, p. 340.) Natural infections could be counted on in the greenhouse cases, because the plants were set close together and intermixed, and in addition a thorough syringing with a strong water pressure was applied whenever the plants were watered. No infections occurred on any of the rather remote wild relatives of Citrus until several weeks after the last inoculation. This may be due either to the resistance of the plants and the subsequent period of accommodation of the organism to the host or to the rather extended period of incubation. The last statement appears to be more nearly correct and is substantiated by Lee's results.

Of the rutaceous plants not closely related to the genus Citrus positive infections have been obtained on *Casimiroa edulis*, *Chalcas exotica*, and *Claucena lansium*. Lee has successfully inoculated the last two plants and, in addition, *Evodia ridleyei*, *E. latifolia*, *Melicope triphylla*, and *Toddalia asiatica*. Both of us have failed to produce any infection on *Xanthoxylum* spp., while so far *Glycosmis pentaphylla* has remained immune.

Jehle (*1*, *2*) reports successful needle-prick inoculations on *Xanthoxylum fagara* (L.) Sarg. and *X. clava-hercules* (L.) Sarg. He also obtained watery swelling on *Chalcas* (*Murraea*) *exotica*. In all cases, a few nontypical, unruptured spots have been produced at wounds or scratches only. (Pl. 59.) Of the plants infected, *Chalcas exotica* responds the least, and the period of incubation is long. Lee likewise obtained only a very weak reaction.

No doubt other plants widely removed from the genus Citrus will be successfully inoculated under certain conditions, although it is extremely doubtful if any of the plants in this group will prove susceptible enough to warrant any attention except to be of interest from a scientific standpoint.

In the subtribe Aeglinae, of the tribe Citreae, *Chaetospermum glutinosum* is susceptible enough to rank with some of the Citrus fruits in

susceptibility. This confirms the observations of Lee (4) in the Philippines, where he has found Chaetospermum generally infected under field conditions. The spots (Pl. 58, C) produced on this plant are ruptured, corky, and more or less typical of those found on the plants in the genus Citrus. They occur on the leaves in the absence of wounds. *C. glutinosum* is the most distantly removed relative so far found which is quite susceptible and produces canker spots typical of those found on *Citrus* spp.

It is rather peculiar that the other plants tested in this subtribe are immune or nearly so, especially Balsamocitrus and Aegiopsis. A few small, nontypical, unruptured spots have been found on *Aegle marmelos*, but only at wounds. Thus Aegle can be classed with the first group discussed in its resistance to canker. Lee (4) failed to obtain any infection on Aegle. This is the only plant of the whole group tested by us where Lee's results and mine failed to check.

On both *Feronia limonia* and *Feroniella lucida* positive infections have been obtained. While the spots are typical of those described for the rutaceous group, they can develop in the absence of wounds on the leaves.

Of the plants tested in the subtribe Lavanginae, *Triphasia trifolia* and *Severinia buxifolia* have remained free from canker. Lee (4) has likewise failed to obtain infection on these plants after repeated trials. No doubt, both species will prove immune to canker. *Hesperthusa crenulata*, on the other hand, is quite susceptible, in fact, almost as much so as Chaetospermum. While both leaves and twigs are attacked in the absence of wounds, the spots (Pl. 59, B) do not resemble those found on any other host. They are flat, and though they rupture, there is no evidence of the corky tissue so typical of the canker spots on Citrus.

Citropsis Schweinfurthii, in the subtribe Citrinae, ranks with the rutaceous plants in susceptibility, in that infections occur only at wounds and the spots are small, nontypical, and unruptured. *Atalantia citrioides* and *A. ceylonica* have proved quite susceptible. The spots (Pl. 59, A) are medium-sized, ruptured, corky, and resemble somewhat those found on Chaetospermum. Lee reports *A. citrioides* and *A. disticha* rather resistant.

Poncirus trifoliata is without doubt the most susceptible of the wild relatives. Somewhat less susceptible is *Eremocitrus glauca*. Canker spots (Pl. 59, D) have appeared on the leaves, thorns, branches, and stems of this plant. The spots are small but ruptured and corky, while on the branches they are of a girdling type, resembling the stem cankers on *P. trifoliata*, except in size. Equally susceptible and with the same type of canker spots are *Microcitrus australis* and *M. Garrowayi*. *M. australasica* and its variety *sanguinea* are more resistant, although spots of the same type occur on the leaves, thorns, and twigs.

Of the kumquats, *Fortunella Hindsii* is susceptible. Lee (4) reports that canker occurs naturally on the wild plants on the mountains near Hongkong. The canker spots (Pl. 60, C) on *F. Hindsii* are ruptured,

raised, and corky, resembling those found on Citrus. The other three species of kumquats tested are equally resistant. While infection has been more or less general in the greenhouse on the young foliage the spots with but few exceptions have remained unruptured.

Thus, outside the subtribe Citrinae, only two susceptible plants, *Hesperthusa crenulata* and *Chaetospermum glutinosum*, have been found under greenhouse conditions. The rest of the plants which were successfully inoculated all produced nontypical, unruptured spots at wounds. To this group can be added *Citropsis Schweinfurthii*. The plants reported free from canker will probably remain immune, while other plants not tested may prove susceptible when inoculated. The remaining plants in the subtribe Citrinae have all been successfully inoculated.

In the field successful inoculation both natural and artificial have been produced on *Hesperthusa crenulata, Poncirus trifoliata, Fortunella Hindsii, F. margarita, F. japonica, Microcitrus australasica*, and *M. australis*. Of these *P. trifoliata* and *M. australis* are very susceptible. No doubt under favorable conditions *Atalantia citrioides, A. ceylonica, Eremocitrus glauca*, and *Chaetospermum glutinosum* can be successfully inoculated in the field. However, none of them will prove as susceptible as *P. trifoliata*.

Thus, only the relatives most susceptible under greenhouse conditions have been successfully inoculated in the field. So far as the menace of citrus-canker to the Citrus industry in the United States is concerned, with the exception of *Poncirus trifoliata*, none of the relatives, native or introduced, discussed above are susceptible enough to warrant further attention.

The index of susceptibility to citrus-canker of these plants should be based not on the ability to successfully produce canker infections through needle pricks or wounds, under abnormal conditions, but rather on the ability of the organism to gain entrance into the tissues through natural openings of the leaves in the absence of both artificial and natural wounds. Therefore, the senior author believes that even though he has been able to inoculate a large number of the wild relatives the results have no bearing on the eradication program. It is purely of scientific interest to know that *P. citri* is not limited to the genus Citrus but can produce, under certain conditions, infections on a wide range of plants in the family Rutaceae to which Citrus belongs.

The senior author has devoted considerable attention to a study of the various types of spots produced on the various hosts, hoping to be able to correlate the type of spot with resistance. In brief, the spots as observed on the relatives can be classed as follows: Small, slow growing, nontypical, unruptured spots (Pl. 57) occurring only at wounds on rutaceous plants; same type of spots, but occurring on the leaves in the absence of wounds (on *Feronia limonia*); more typical spots (Pl. 60, A) which are unruptured except at wounds (on *Fortunella margarita*); and typical, ruptured, corky spots (Pl. 63, D) (on *Poncirus trifoliata*.)

SUSCEPTIBILITY OF CITRUS FRUITS

Citrus hystrix DC. (CPB 7872 and 2881, seedlings), I, II, III, in field, 1917 and 1918. "Cabayao" (CPB 7831, seedlings), I, II.

Two types of these plants have been tested. One group has pointed leaves while the second has rounded ends. Very little infection has been found on the plants with the pointed leaves, either in the field or the greenhouse (Pl. 61). However, 70 to 100 per cent of the leaves having rounded tips were infected with small to large and scattering to many spots. Some defoliation occurred. Rather large spots of a girdling type are common on thorns, twigs, branches, and old wood.

Lee (3) finds that of the numbers tested by him in the Philippines seven were severely infected, three moderately so, one slightly, while canker was not observed on four, and one proved resistant. As the group is obscure, although a large one, some forms may be found resistant to canker. However, the majority, especially those with rounded leaves, will prove to be almost as susceptible as grapefruit.

Citrus medica L. Citron of commerce (CPB 7768 and 7836, cuttings and seedlings), I, II, and III. "Sidro" (CPB 7816, seedlings), II. "Nana" (CPB 11281, seedlings), II, III. "Odorata" (CPB 11294, seedlings), II, III. "Etrog" (CPB 11178, seedling), I,[1] II.

Of the citrons tested, the "Etrog" proved to be the most susceptible. All the leaves were infected and some defoliation occurred. Twig and stem infections were also present. A few twig and stem spots were found on CPB 7768, 7836, and 11281. On the other numbers canker was limited to the foliage, the percentage of leaf infection varying from 30 to 100, with little defoliation.

The spots were, for the most part, small and scattering and very distinct in character. No doubt the texture of the leaves has a direct influence on the type of spot produced and also on the susceptibility of the leaves. The citrons, as a whole, while rather easily infected, are not as susceptible as grapefruit but are more so than Satsuma, (*Citrus nobilis* var. *unshiu* Swingle).

Lee (3) tested 14 numbers in the Philippines and found 1 resistant, 5 on which canker was not observed, 5 with medium infection, and 3 severely infected. He is of the opinion that some of the citrons may be considered as canker-resistant.

Citrus sp. Small lemon (CPB 7833, seedlings), I,[1] II. Sweet lemon (CPB 1158, seedlings), I, II. "Davo lemon" (CPB 7837, seedlings), II, III. Limon real 18 (CPB 7819, seedling), II.

The plants of the lemon group so far tested have all proved more susceptible than the citrons. All the plants in the experiments have few to many large twig and stem spots, while 50 to 100 per cent of the leaves are infected. Canker also caused some defoliation of the plants.

Two types of spots are produced on the foliage. Where the texture of the leaf resembles that of the citron the same kind of spot is produced, except that it is larger. On the plants with smooth leaves the spots resemble those found on grapefruit (Pl. 62). In the scale of susceptibility, the lemons so far tested rank just below grapefruit.

Lee's results (3) also show that the lemons are fairly susceptible under Philippine conditions.

Citrus sp. Ichang lemon (CPB 11291 and 11204, seedlings), I, II, III, and I, II,[1] III, in field, 1918.

The Ichang lemon was not considered under the lemon group because it appears to be a natural hybrid, possibly betweeh lemon and pummelo. The plants are very susceptible, for from 30 to 100 per cent of the leaves are infected and several plants have severe twig and stem infections. All three plants in the isolation field were reported infected during September, 1918, and August, 1919. However, all spots were localized on the leaves.

[1] Included in experiments of March 21, 1918.

The spots resemble those on grapefruit leaves (Pl. 62), but the plants rank with the lemons in susceptibility to canker.

Citrus aurantifolia (Auct.) Swingle (*C. limetta* Auct., not Risso). Sour lime (CPB 7338, seedlings), I, II.

Only a small percentage of the leaves are infected with canker. The spots are also very small and scattering. No twig or stem infections have ever developed.

The spots (Pl. 60, E) resemble those on citron to some extent. However, they are smaller, more compact, less corky, and darker in color. While the plants are rather easily infected, the spots increase slowly in size and are few in number. The sour lime is much more resistant than either the citrons or lemons, almost approaching Satsuma in resistance. Lee (*3*) reports that the limes, with the possible exception of the "Tahiti," are very susceptible in the Philippines.

Citrus grandis (L.) Osbeck (*C. decumana* L.). Grapefruit (CPB 11170, 7834 and Duncan (Alabama), seedlings), I, II; I, II; and II, III. Grapefruit (Duncan budded on *Poncirus trifoliata*), I, II. Chinese pummelo (CPB 11061, seedlings), I, III. Hirado Buntan(?) pummelo (CPB 7993, seedlings), I, II. Indian pummelo (CPB 11166 and 2968A, seedlings), I, III, and I, III. Siamese pummelo (CPB 11201 and 6111, seedlings and on *C. Schweinfurthii*), I, III, and II.

With possibly two exceptions, all the grapefruit plants tested in the greenhouse have proved to be extremely susceptible. Approximately 100 per cent leaf infection occurred, with considerable defoliation. Twig and stem infections were also severe, the spots being large and of a girdling type. Several shoots have been killed by the girdling spots.

The "Hirado Buntan," reported very susceptible in the preliminary report, has stood up very well, and at the November, 1918, reading only 5 to 10 per cent of the leaves were infected, with few or no spots on the twigs. The Siamese pummelo, especially the number budded on *Citropsis Schweinfurthii*, shows some resistance to canker.

In the field, severe infections have been obtained on grapefruit seedlings (1919), grapefruit (Duncan) budded on *Poncirus trifoliata* (1917), Sullivan grapefruit (CPB 11001 and 11054) (1918), Mark's Chinese pummelo (CPB 11061, 11217F, and 11217G) (1918), Roeding's Indian pummelo (CPB 2968A and 11166) (1918), Florida Shaddock (CPB 11255) (1918), Orangedale Chinese pummelo (CPB 11212 U) (1918), French Martin's Chinese pummelo (CPB 11213 J) (1919), and pummelo (CPB 11219 I) (1918). (See Pl. 62.)

Only slight leaf infections have been obtained on the Hirado Buntan pummelo (CPB 7993 and 11021) and recently on the Siamese pummelo (CPB 11201 and 6111) although these plants have been in the isolation field for the past two seasons and surrounded by badly infected plants.

Mr. Swingle reports that in Japan the Hirado Buntan is quite resistant, whereas Lee (*3*) states that the Siamese pummelo is the only variety of *Citrus grandis* tested by him which gives any promise of being resistant.

Citrus sinensis Osbeck (*C. Aurantium* Lour. and Auct. not Linn.). Grenadine orange (CPB 7773, seedlings), I,[1] III. Parson Brown orange (CPB 11324, seedlings), I,[1] III. "Naranja" (CPB 7929, seedlings), II, in field, 1918. Orange (CPB 66A seedlings), I,[1] III.

With the exception of a few small, scattering spots on the twigs of two plants, canker is limited to the foliage of the plants in this group. Apparently the Parson Brown orange is the most susceptible, followed by CPB 66A. The "Naranja" and Grenadine oranges are somewhat more resistant in that only a small percentage of the leaves are infected, the spots are fewer and smaller, and no twig infections are present.

[1] Included in experiments of March 21, 1918.

All the plants of this group tested in the isolation field have been successfully inoculated. The following numbers were represented: CPB 11196 Narute, CPB 11164 Temple, CPB 11028 South Carolina, CPB 11198 Japanese No. 1, and CPB 11199 Japanese No. 2. The spots in all cases resembled those found on grapefruit.

In susceptibility this group ranks just above the citrons in that twig and stem infections are of more general occurrence. Lee (*3*) has observed that the Mediterranean varieties are less susceptible than the others. This fact has also been pointed out by other investigators.

Citrus nobilis Lour. King of Siam (CPB 2105, seedlings), I, III. "Naranjita" (?) (CPB 7830, seedlings), II, III, var. *deliciosa* Swingle. Tangarine (CPB 11195, seedlings), I, III. Cleopatra tangerine (CPB 11338, seedlings), I, II, III, var. *unshiu* Swingle. Satsuma (on *Poncirus trifoliata* Alabama), II, III.

Twig infection, consisting of small, unruptured, scattering spots, is limited to one plant (Naranjita) of this group. The spots (Pl. 63, B, at left) on the leaves are small to medium-sized, and, as a rule, rather scattering. The King of Siam orange is apparently the most susceptible. The Satsuma plants are the most resistant.

The spots (Pl. 63, B) found on the plants of the *Citrus nobilis* group are very characteristic, resembling to some extent those produced on kumquats. They are of medium size, dark, raised, compact, mostly unruptured, with a distinctly oily outline and some yellow zone. Ruptured spots are only slightly corky.

Recent investigations by Tanaka (*9*) and Scott (*8*) show that there are a number of distinct strains of Satsuma in Japan, of which three have been found in Alabama. Experiments are now under way to determine the relative susceptibility and resistance of these strains under field conditions. Successful inoculations have been made in the field on Satsuma (Pl. 64) and the Cleopatra tangerine. However, these plants are not easily attacked, canker being limited to the foliage.

All the plants tested in this group are very resistant. The writers believe that under field conditions suitable for Satsuma culture, and with no interplanting of susceptible varieties, this orange can be grown with little or no loss from canker even when the disease is prevalent. From the results so far obtained all the plants of the *Citrus nobilis* group can be placed in this class. In the investigations on susceptibility and resistance any variety showing as much resistance to canker as the Satsuma has been classed as promising.

Citrus mitis Blanco. Calamondin orange (CPB 11265, 44305, and 7065 seedlings), I, II,[1] II, III, and I,[1] II,[1] III, in field, 1917, 1918, and 1919.

Scattering stem infections and some defoliation have occurred on two of the seven plants tested. From 20 to 90 per cent of the foliage of the other plants have small to large, scattering spots.

The spots (Pl. 63, E) are altogether characteristic, and for the most part are unruptured. They are round, raised, compact, and oily, somewhat like the spots described for kumquat. When ruptured the spots are flat and have very little cork.

In the field canker is limited to the foliage, and the plants are more resistant than Satsuma. Lee (*3*) also finds that in the open *Citrus mitis* is truly resistant, and he thinks that it is apparently more so than Satsuma.

Citrus sp. Kansu (Yuzu) orange (CPB 11242, seedlings), I,[1] II, III, in field, 1918 and 1919.

The Kansu orange, collected by Mr. Frank N. Meyer in north China several years ago, is considered by Dr. T. Tanaka[2] to be the same as the well-known "Yuzu" used in Japan for many years as a stock.

Under both field and greenhouse conditions the plants have proved resistant. Apparently the leaves are quite easily infected, but the spots rarely increase in size,

[1] Included in experiments of March 21, 1918.

[2] The data are unpublished.

although they commonly rupture (Pl. 60, D). The spots do not penetrate to the upper surface. The plants are much more resistant than *Poncirus trifoliata*, which the writers understand has to a large extent replaced Yuzu as stock for Satsuma in Japan. Other conditions being equal, Yuzu is to be preferred to *P. trifoliata* from the standpoint of canker susceptibility.

Citrus sp. Natsu-mikan (CPB 11184, seedlings), I, II, III, in field, 1918 and 1919.

In some ways this plant resembles the hybrids between the grapefruit and loose-skinned oranges, such as the tangerine, known in this country as tangelos.

All plants of the Natsu-mikan in the greenhouse and field have been rather severely infected. Some twig and stem infections have been found, and from 50 to 100 per cent of the leaves have medium to large and scattering to many spots. The spots, although larger and more corky, resemble those found on Satsuma. Some defoliation has taken place, due to canker.

If the Natsu-mikan is closely related to the mandarin orange it is very much more susceptible than any of the plants so far studied in this group. Lee (*3*) reports the Natsu-mikan as susceptible in the Philippines.

Citrus excelsa Wester. (CPB 11280, seedlings), I, III.

From 90 to 100 per cent of the foliage of the two plants is infected with many large spots. Some few spots on the twigs are also present. Because of the citron-like texture of the leaves, the spots resemble those on the citron, except in size. Apparently it is not quite as susceptible as grapefruit.

In the Citrus fruits, where so many species and varieties were tested with more or less varying results, it is extremely hard to classify the susceptibility of these plants, especially where so many factors must be taken into consideration. Probably the most important and vexing factor is the physiological condition of the plant. In looking over the notes taken approximately each month on the plants in the experiments, it is found that there are certain cycles of canker infection which coincide with the growth periods of the plants. Thus, one or two observations on inoculated plants in the greenhouse or on those exposed to natural infection in the field are not sufficient to determine accurately the exact susceptibility or resistance of a plant. Some of the points to be reckoned with under the factor of the physiological condition of the plant are the rate of growth, not only of the plant but of the leaves themselves, age and size of the plant and leaves, leaf texture, and rate of maturation of the leaves. All these have an important relation to canker susceptibility and resistance.

Leaf texture with its various ramifications probably plays an important rôle in determining resistance in many cases. This can be best illustrated by comparing an infected kumquat leaf (Pl. 60, A) with an old grapefruit leaf (Pl. 60, B). The leaves are apparently very similar in texture, and a close study of the spots produced on the two shows that they are identical. In other words, while an ordinary grapefruit leaf is still thin and light green in color, it is very susceptible, large corky spots being produced. However, if an old leaf is taken which has apparently the same texture as a leaf of the kumquat, it is as hard to infect as the kumquat, and small, rounded, glistening spots are formed.

When no twig or stem infection occurs and only small, scattering, unruptured spots appear on the leaves, the plants show enough resistance to be classed as resistant. An intermediate group can be formed where the spots are larger, ruptured, and more general on the leaves, with occasional twig and stem infections. Plants placed in this group might be found promising under certain conditions. Plants which show large, ruptured spots on the leaves, severe enough to cause defoliation, and large girdling cankers on the twigs and stem should be classed as extremely susceptible. With these remarks in mind, the writers will attempt to rank the Citrus fruits, provisionally, in groups according to their susceptibility to citrus-canker.

The plants of the grapefruit and pummelo group are extremely susceptible. However, the Siamese and possibly the Hirado Buntan pummelos give promise of showing some resistance to canker.

Of the numbers tested belonging to *Citrus hystrix*, those with rounded leaves are as susceptible as grapefruit. The plants with pointed leaves are apparently more resistant to canker. More study is needed to determine whether this will hold for all forms of *Citrus hystrix* in the Philippines.

All numbers of the lemons tested, including the Ichang lemon, show about equal susceptibility, which is slightly less than that of grapefruit.

The plants of the sweet-orange group vary somewhat in susceptibility. Leaf infections are severe, and twig and stem cankers are common. As a whole, they are not as susceptible as the lemons.

Citrus excelsa and the Natsu-mikan are equally as susceptible as the plants of the sweet orange group.

Since only one number of the limes was tested, the position which the limes should take in the scale of susceptibility is doubtful. The sour lime tested proved to be somewhat resistant. However, Lee finds that with one exception the limes are susceptible.

While the citrons tested are easily infected, the spots are small, increasing very slowly in size. Twig infection occurs only occasionally and is the exception rather than the rule.

Citrus mitis, at least seedling plants such as were used in the inoculation experiments in the greenhouse, while showing some resistance are more susceptible than in the field. Leaf infections are scattering, and twig cankers are rarely produced.

The Kansu (Yuzu) orange so far has proved decidedly resistant. No twig cankers have occurred, and only small, scattering spots have developed on the foliage.

All numbers of the *Citrus nobilis* group tested have proved to be decidedly resistant, and, no doubt, all of these plants, if not mixed with susceptible varieties, could be grown under canker conditions. That does not mean that it would be economical or at all advisable to allow canker to persist even in unmixed plantings of Satsuma or other varieties of *C. nobilis*.

SUSCEPTIBILITY OF CITRUS HYBRIDS

Faustrime[1] (*Citrus aurantifolia*, West Indian lime, × *Microcitrus australasica*). (CPB 49819, 49823, and 49835, cuttings), II; II and III.

Faustrimon (*Citrus limonia*, lemon, × *Microcitrus australasica*). (CPB 49841, 49843, and 49844, cuttings), II; II and III.

Faustrimedin (*Citrus mitis*, calamondin, × *Microcitrus australasica*). (CPB 47431, cuttings), I, III, in field, 1918.

From 30 to 90 per cent of the foliage of these plants is infected with scattering spots (Pl. 63, A). Some defoliation from canker has taken place. Thorn, twig, and stem cankers are common (Pl. 63, A). The spots are similar to those found on *Microcitrus australasica*, except that they are larger and more ruptured. The spots on the twigs and stem are of a girdling nature. The last number has been tested in the field with positive results on the foliage only. These hybrids are more susceptible than *M. australasica*.

Citrange (*Poncirus trifoliata*, × *Citrus sinensis*). Seedlings.

Two or more plants of each number of the following citranges have been given a thorough test in both the greenhouse and the field: Colman (CPB 7896 and 772 AC), Rusk (CPB 7956, 11030, 7895, and 716), Rustic (CPB 7934 A), Sanford (CPB 7963 and 1423 AB), Savage (CPB 7961 and 1423 AB), Willits (CPB 7897 B, 7960, and 777 AB), Etonia (CPB 749 AB), and citranges (CPB 1425 AB, 1416, 43931, 43480, and 43434).

The percentage of leaf infection has varied from 10 to 100 per cent, depending on the condition of the plant. The majority showed over 50 per cent of infected leaves. Defoliation of the leaves due to canker was common. Large girdling spots have appeared on the stems of most of the plants. The spots (Pl. 63, D) on the leaves and twigs are similar to those produced on *Poncirus trifoliata*.

While some variations have occurred in the susceptibility of the different numbers, the results as a whole show that all the citranges (Pl. 65, B) are equally as susceptible as *Poncirus trifoliata* (Pl. 65, B). Thus none of the citranges tested are of any promise in the search for a resistant stock.

Citrumelo (*Citrus grandis*, Bowen grapefruit, × *Poncirus trifoliata*). (CPB 4493, 4554, 4564, and 4475, seedlings), I, II; I, II (2 plants); I and I, III.

Almost 100 per cent leaf infection, with some defoliation, occurred on all the plants. The spots (Pl. 63, D) are large, scattering to many, and resemble those produced on *Poncirus trifoliata* except in size. Girdling spots of various sizes occurred on most of the plants, not only on the twigs and branches but even on the old wood.

The citrumelos (Pl. 65, C) are even more susceptible than *Poncirus trifoliata* and, therefore, are of no economic value from the standpoint of their behavior to citrus-canker.

Citradia (*Poncirus trifoliata*, × *Citrus aurantium*, sour orange). (CPB 50850, seedlings), I, II.

While from 40 to 80 per cent of the leaves have been infected with small, scattering, typical spots, no spots have been produced on the twigs or branches. Apparently, the citradia (Pl. 65, D) is a slower grower than the rest of the *Poncirus trifoliata* hybrids. The susceptibility of these plants, however, is sufficient to bar them from further tests.

Citrandarin (*Citrus nobilis* × *Poncirus trifoliata*). Seedlings.

In the greenhouse, plants of the following numbers have been given a thorough trial. CPB 40210, 40303, 40315, 40368 B, and 48529. All of these numbers are hybrids

[1] The hybrids were supplied by Mr. Walter T. Swingle, who informs me that they were labeled with the aboratory names, for the most part still unpublished. Citrange, limequat, and tangelo have been published, but citrunshu, cicitrange, citrumelo, citradia, citrandarin, faustrime, faustrimon, faustrimedin, citrangedin, citrangarin, citranguma, citrangequat, limelo, bigaraldin, orangelo, orangequat, clemelo, siamelo, satsumelo, siamor, calarin, and calashu are tentative laboratory names that may not be used in the reports which may later be issued concerning hybrids. Many hybrids which in this paper are given separate names will in later reports be grouped under some one name.

of the King of Siam orange with *Poncirus trifoliata*. In the field, CPB numbers 40175 A, 49720, 49721, 49722, 49724, and 49726 (cross between King of Siam orange and *Poncirus trifoliata*), 49624, 49625, 49629, 49644, 49663, and 49644 (cross between Clementine orange and *P. trifoliata*), 49686, 49688, 49695, 49699, and 49712 (cross between Oneco tangerine and *P. trifoliata*), 49732, 49735, 49737, 49746, and 49748 (cross between a tangerine and *P. trifoliata*) were tested.

Some individual variation in susceptibility due to the condition of the plants occurred in the greenhouse. However, all plants proved susceptible. From 30 to 100 per cent leaf infection, with some defoliation, was observed on the majority of the plants. Some scattering twig infections occurred on all but one number. Rather large, girdling spots on the old wood were found on several of the plants.

In the isolation field, all the plants have been successfully infected. An abundance of spots occurred not only on the leaves but on the twigs, branches, and old wood. No differences were noted in the susceptibility of the plants having different *Citrus nobilis* varieties as one parent. The *Poncirus trifoliata* blood predominates, in that all the leaves of the above numbers are like this plant and all have the same leaf texture. All the citrandarins (Pl. 65, E) are about as susceptible as their parent, *P. trifoliata*.

Citrunshu (*Citrus nobilis* var. *unshiu*, Satsuma, × *Poncirus trifoliata*). Seedlings and on *P. trifoliata*.

These plants are very similar to the citrandarins, and their behavior towards citrus-canker is likewise the same. Of the nine numbers (CPB 51102, 49607, 49608, 49611, 49615, 49616, 49619, 49620, and 49623) tested in the field, all proved equally susceptible. Leaf infection was common, and some stem cankers were present. They are more resistant than the citrandarins, although further tests may show them to be as susceptible. The type of spot produced on all these plants is identical to those on *Poncirus trifoliata*.

Cicitrange (*Poncirus trifoliata* × Colman citrange, and *P. trifoliata* × Sanford citrange). (CPB 48290 and 48316A, seedlings), I, II, and I, II, III.

These plants have shown considerable susceptibility to canker throughout the course of the inoculation experiments; in fact, one plant was killed by canker, while the others have been severely attacked. Without question, the cicitranges (Pl. 65, F) are equally as susceptible as *Poncirus trifoliata*.

Citrangedin (a citrange × *Citrus mitis*, calamondin). Seedlings and on *Poncirus trifoliata*.

All plants in the greenhouse (CPB 48045) and isolation field (CPB 50485, 50486, 50493, 50495, 50500, and 50501) have been successfully infected. The spots are rather small and scattering on the leaves. Few twig and stem cankers have been observed. The spots are not typical of those produced on the citranges but resemble more those on *Citrus mitis*. The fact that these plants are more resistant to canker and that the spots themselves are not similar to those on the citranges can be traced back primarily to a difference in the leaf texture of the two hybrids. The citrangedins are more susceptible than *C. mitis*, but they are more resistant than the citranges. While the leaves still retain their trifoliate character, the size, shape, and texture of the leaflets are different. They are also a darker green, and apparently mature faster than the leaves of the citranges.

Citrangarin (Sanford citrange × *Citrus nobilis* var. *deliciosa*, Oneco tangerine). Seedlings.

A plant (CPB 48776) of this hybrid was tested in the isolation field and has been successfully infected with a few scattering spots resembling those on Satsuma. While the leaves of this plant are trifoliate, they have a texture similar to that of the

tangerine. It is interesting to compare the susceptibility of the citrangarin and the citrandarin. The latter was found to be as susceptible as *Poncirus trifoliata* and was very similar in character. The citrangarin, on the other hand, while it retains the trifoliate character, has a leaf texture more like the second parent and is much more resistant.

Citranguma (*Citrus nobilis* var. *unshiu*, Satsuma, × Morton citrange). Seedlings.

The citranguma (Pl. 65, G) is possibly slightly more susceptible than the Satsuma. Leaf infections have been secured in both the greenhouse (CPB 48055, and 48055A) and field (CPB 49773). An occasional spot has been found on the smaller twigs of these plants in the greenhouse and field.

The leaf texture and type of spot are similar to those found on the Satsuma. However, the leaves do not reach maturity so soon. There is a decided tendency in the citranguma plants for the leaves to revert from the trifoliate to a single leaf. This is especially noticeable on the new growth.

Citrangequat (Willits citrange × *Fortunella margarita*, oval kumquat). Seedlings.

The citrangequat, without question, is the most promising hybrid so far tested. No natural infections have ever been obtained in the field (CPB 48010E and 48010F). Under greenhouse conditions (CPB 48010 and 48010D) only several tender leaves have been infected with tiny, compact, unruptured spots (Pl. 63, C). So far, the citrangequat has shown more resistance than any of its parents.

These plants (Pl. 65, H) make a splendid, rapid, straight growth. The rate of growth is more rapid than that of *Poncirus trifoliata*, and the plants are much better adapted for budding purposes. The trifoliate leaves are rather small and retain considerable of the leaf texture of the kumquat. The maturation of the leaves is also as rapid as in the kumquat. The leaves, especially those on the new growth, have a tendency to revert to the signle leaf of the kumquat.

Limequat (*Citrus aurantifolia*, West Indian lime, × *Fortunella japonica*, round kumquat). Seedlings.

All plants in the greenhouse (CPB 48787A, 48787B, 49787E, 49792E, and 48798E) and isolation field (CPB 48792E) have been infected. Leaf, twig, thorn, branch, and stem spots are common. In fact the limequat is more susceptible than either parent.

Several plants have died in the greenhouse experiments from overwatering, although plants next to them have thrived. On the whole, the limequat plants (Pl. 66, B) worked with have not been strong nor altogether healthy. The rate of growth of these plants has also been slow. It may be that strong, healthy plants growing under ideal conditions would show more resistance to canker. However, judging from the results obtained with the plants available, the limequat is somewhat susceptible.

Limelo (*Citrus aurantifolia*, West Indian lime, × *C. grandis*, sour pummelo). (CPB 40502, 40526A, and 40567B, seedlings), I, II; I, III, and I, II.

All limelos (Pl. 66, A) tested have proved to be equally as susceptible as grapefruit, so that they are of no practical importance from their ability to resist canker under orchard conditions.

Bigaraldin (*Citrus aurantium*, sour orange, × *C. mitis*, calamondin). On *Poncirus trifoliata*.

Only one plant (CPB 50352) of this hybrid was included in the field. It was successfully infected and no doubt will prove as susceptible as the sour orange.

Orangelo (*Citrus grandis*, Bowen grapefruit, × *C. sinensis*, Lamb summer orange). Seedlings.

All three plants (CPB 1668A) in the isolation field have been successfully infected. Spots are limited to the leaves. No twig or stem cankers have developed. Judging from the type of spot produced and the leaf texture, this hybrid will prove rather susceptible.

Orangequat (*Citrus sinensis*, Hartley mandarin, × *Fortunella margarita*, oval kumquat). On *Poncirus trifoliata*.

The one plant (CPB 50312) in the isolation field was easily infected. A few twig and stem cankers have appeared. Judging from the leaf texture and type of spot, it may prove susceptible.

Clemelo (*Citrus nobilis*, Clementine orange, × *C. grandis*, grapefruit). On *Poncirus trifoliata*.

Both the plants in the greenhouse (CPB 49006, 49012, 49013, 49025, and 49038) and those in the field (CPB 49029, 49032, and 49049) have been easily infected. From 50 to 100 per cent leaf infection with some defoliation has occurred. Most of the plants in the greenhouse have large, girdling stem cankers. The spots in all cases resemble those on grapefruit. The clemelos (Pl. 67, B, at left) are as susceptible as grapefruit and therefore can not be considered promising.

Siamelo (*Citrus nobilis*, King of Siam orange, × *C. grandis*, grapefruit). Seedlings and on *C. grandis*.

The behavior of the siamelos (Pl. 67, A) (CPB 47255B, 51588, 51598, 59852, 52007N2, 52013S9, and 52021A5) in the greenhouse, and 50320, 52007N2, 52007B3, and 51947 in the field, toward canker is about like that of the clemelos. However, they are not quite as susceptible, in that less twig and stem infections occurred.

Satsumelo (*Citrus nobilis* var. *unshiu*, Satsuma, × *C. grandis*, grapefruit.) On *Poncirus trifoliata* and *C. grandis*.

Many numbers of this hybrid have been tested in the field (CPB 50304) and greenhouse (CPB 50304, 52009A 2, 52009G 5, 52011, and 52011G 4). Thus far, the satsumelos react to citrus-canker in the same manner and extent as the siamelos (Pl. 67, B, center).

Siamor (*Citrus nobilis*, King of Siam orange, × *C. sinensis*. (CPB 52029E 2, seedling), III.

The plant included in the greenhouse experiment has proved to be extremely susceptible to canker Heavy defoliation and severe stem cankers occurred soon after inoculation. Leaf texture and the type of spot are identical with those of grapefruit. The plant is likewise as susceptible.

Tangelo (*Citrus nobilis* var. *deliciosa*, tangerine, × *C. grandis*, Florida grapefruit). Seedlings, and on *C. grandis*.

Besides the Thornton (CPB L715, and 11034 greenhouse and L714B field) and Sampson (CPB L789A and 7664 greenhouse, and 7664 and 11037 field) tangelos, which are now being grown to a small extent in Florida, about a dozen numbers (CPB 1230, 1257A, L16, 7161, 7675C, 1191, 1348A, 1262B, 40971A, 52018C2, and 52018E2 in the greenhouse and CPB 52016E4 in the field) were tested. Plants of all numbers have been infected. From 20 to 100 per cent leaf infection has occurred in the greenhouse. Twigs and stem infections have not been general but are rather the exception than the rule. In the field, canker has been limited to the foliage. The spots in all cases resemble those on grapefruit. Careful observations have shown that those plants (Pl. 68, B) with leaves resembling the tangerine are more resistant than the numbers with grapefruit leaves. For the present the tangelos can be considered somewhat promising but not quite so resistant as the Satsuma.

Calarin (*Citrus mitis*, calamondin, × *C. nobilis* var. *deliciosa*, tangerine). On *Poncirus trifoliata*.

The one plant (CPB 50314) tested proved to be easily infected in the field. However, no twig or stem spots have been observed. Further trials are necessary before the susceptibility of the plant can be definitely judged.

Calashu (*Citrus mitis*, calamondin, × *C. nobilis* var. *unshiu*, Satsuma.) On *Poncirus trifoliata*.

The plant (CPB 50309) in the isolation field has reacted to canker in about the same degree as the calarin.

Among the numerous crosses of Citrus fruits made by Mr. Swingle *Poncirus trifoliata* was used as one parent in the hope of obtaining hybrids more resistant to low temperatures than the ordinary Citrus fruits. Thus, *P. trifoliata* has been crossed with sweet orange, sour orange, grapefruit, King of Siam orange, tangerine, Clementine orange, Satsuma, and citrange. While all of the resulting hybrids have proved to be quite resistant to low temperatures they are equally as susceptible to citrus-canker as *P. trifoliata*; in fact, the citrumelos are even more so. The hybrids all resemble *P. trifoliata* in size, shape, and texture of leaves, so that the more or less resistant mandarin group has had no influence on the character or resistance of the hybrid. Thus, it can be safely predicted that all crosses with *P. trifoliata* will yield a hardy hybrid resembling *P. trifoliata* and equally susceptible.

On the other hand, the citranges, while equally as susceptible as *Poncirus trifoliata*, when crossed with calamondin, tangerine, Satsuma, and kumquat, yield hybrids which are hardy and at the same time resistant to citrus-canker. In fact, the citrangequat is even more resistant than the kumquats themselves, in spite of the fact that it is a rather rank and rapid grower. The citrangarins and citranguma, while not as resistant as the citrangequat, are decidedly more resistant than the citrandarin and citrunshu, the corresponding hybrids with *P. trifoliata*.

These hybrids, while still retaining the trifoliate character of *Poncirus trifoliata*, are more like the resistant parent in texture of the leaves. In the case of the citranguma and citrangequats there is also a tendency for the leaves to revert to a single leaf. Thus, any plant resistant to canker when crossed with the citranges will be hardy and resistant, or even more so than the original resistant parent, as shown by the behavior of the citrangequat, even though the citranges are equally as susceptible to canker as *P. trifoliata*.

The limequat and the orangequat, while more resistant than the lime or orange, are not as resistant to canker as the kumquat. The leaves resemble those of lime and orange, respectively, in size and shape, but the leaf texture resembles more that of the kumquat. The citrangequat is much more resistant to canker that the two hybrids named above, in spite of the fact that the citrange is much more susceptible than either the lime or orange.

The influence of pummelo on the second parent varies. As is to be expected, pummelo crossed with lime and orange produced hybrids which are as susceptible as grapefruit. Hybrids between pummelo and Clementine orange, King of Siam orange, tangerine, and Satsuma are susceptible to some extent, variation in susceptibility depending more or less on whether the leaf is of the grapefruit or the mandarin type.

The calarin, hybrid between calamondin and tangerine, and the calasshu, hybrid between calamondin and Satsuma, so far give promise of being as resistant as their parents.

FALSE HYBRIDS

. A rather large number of "false hybrids" or nucellar bud sports, the the results of Mr. Swingle's crosses with varieties of Chinese pummelo and American grapefruit were tested in both the field and the greenhouse. The plants were vigorous. growers and produced abundance of new growth. Most of these false hybrids resemble the Chinese pummelo.

All proved extremely susceptible, in fact, even more so than grapefruit (Pl. 68, A). Leaf infections were so severe as to cause defoliation. The spots on the twigs and stems were large and of a girdling nature. A number of twigs and several plants were killed outright by complete girdling. The spots in all cases resembled those found on grapefruit. Thus, all the false hybrids tested are extremely susceptible in both the field and the greenhouse, and no one of them gives any promise of canker resistance.

SUMMARY

(1) The investigations on the susceptibility and resistance to *Pseudomonas citri* Hasse of the wild relatives, Citrus fruits, and hybrids of the genus Citrus reported on in a preliminary paper have been continued in both the field and greenhouse. Many more numbers have been successfully inoculated, others have proved to be extremely susceptible, while some still show considerable resistance.

(2) The successful inoculation of a large number of wild relatives in the greenhouse shows that *Pseudomonas citri* has a wide range of hosts and is not limited to the genus Citrus.

(*a*) Of the rutaceous plants not closely related to Citrus, positive infections have been obtained on *Casimiroa edulis*, *Chalcas exotica*, and *Claucena lansium*. *Xanthoxylum* sp., and *Glycosmis pentaphylla* have remained immune. In all cases a few nontypical, unruptured spots have been produced, but only at wounds or scratches on the leaves. *Chalcas exotica* responded the least, and the period of incubation was long.

(*b*) Of the tribe Citreae, subtribe Aeglinae, *Chaetospermum glutinosum* is the most distantly removed relative, so far found, that is quite susceptible and on which canker spots are more like those found on Citrus. *Aegle marmelos* has been only slightly infected, while *Balsamocitrus Dawei* and *Aeglopsis Chevalieri* have remained immune.

(*c*) Both *Feronia limonia* and *Feroniella lucida* of the subtribe Feroninae have been successfully inoculated. While the spots are typical of those described for the rutaceous group, they can develop in the absence of wounds.

(*d*) Of the plants tested in the subtribe Lavanginae, *Hesperthusa crenulata*, while producing very nontypical spots, is quite susceptible in that infection can take place on the leaves and twigs. *Triphasia trifolia* and *Severinia buxifolia* have remained immune.

(*e*) All plants of the subtribe Citrinae which have been tested have been infected. *Citropsis Schweinfurthii* is weakly positive; *Atalantia citrioides* and *A. ceylonica* are easily infected; *Eremocitrus glauca, Microcitrus australasica, M. australasica* var. *sanguinea, M. australis* and *M. Garrowayi*, plants native to Australia, are rather susceptible in that leaves, twigs, thorns, and branches are attacked; all four species of kumquat have been successfully infected; *Fortunella margarita, F. japonica*, and *F. crassifolia* exhibited considerable resistance, while *F. Hindsii* is very susceptible; and *Poncirus trifoliata* is extremely susceptible.

(*f*) Although working under entirely different conditions and using different methods of inoculating plants, Lee's results with the wild relatives check with those obtained by the senior author in the greenhouse, with one exception.

(*g*) In the field, only the wild relatives which were most susceptible under greenhouse conditions have been successfully inoculated. Of these *Poncirus trifoliata* and *Microcitrus australis* have proved to be susceptible, while *M. australasica* and *Fortunella Hindsii* are somewhat susceptible. *Hesperthusa crenulata* reacts to canker in about the same degree in the field as in the greenhouse.

(*h*) So far as the menace of citrus-canker to the Citrus industry of the United States is concerned, with the exception of *Poncirus trifoliata*, none of the wild relatives, native or introduced, now growing in the Citrus districts are susceptible enough to have any bearing on the eradication program.

(3) Little or no change in the susceptibility or resistance to citrus-canker has been noted among the Citrus fruits from that previously indicated. All plants tested have been successfully inoculated.

(*a*) The plants of the grapefruit and pummelo group are extremely susceptible, with the exception of the Hirado Buntan and Siamese pummelos.

(*b*) Of the numbers tested belonging to *Citrus hystrix*, those with rounded leaves are as susceptible as grapefruit. The plants with pointed leaves are apparently more resistant.

(*c*) All numbers of lemons tested, including the Ichang lemon, show about equal susceptibility, which is slightly less than that for grapefruit.

(*d*) As a whole, the plants of the sweet-orange group are slightly less susceptible than the lemons. *Citrus excelsa* and the Natsu-mikan can be classed with the sweet orange in susceptibility.

(*e*) The sour lime tested proved to be somewhat resistant.

(*f*) While the citrons are easily infected, the spots are small and increase slowly in size. Twig infection is not common.

(*g*) *Citrus mitis*, while showing some resistance, is more susceptible than has been reported from the Philippines.

(*h*) The Kansu (Yuzu) orange has proved somewhat resistant. No twig infection has occurred, and only scattering spots have developed on the foliage.

(*i*) All numbers of *Citrus nobilis* and its varieties have proved to be rather resistant to canker.

(4) All hybrids are attacked by citrus-canker in varying degrees.

(*a*) The citranges, citrumelos, citradias, citrandarins, citrunshus, and cicitranges, all having *Poncirus trifoliata* as one parent, are extremely susceptible. Apparently all crosses with *P. trifoliata* will yield hardy but susceptible hybrids.

(*b*) The citrangedins, citrangarins, citrangumas, and citrangequats, with susceptible citranges as one parent, are not only hardy, but decidedly resistant to canker; in fact, the citrangequat is practically immune in spite of the fact that it is a rapid grower.

(*c*) The limequats and orangequats are somewhat susceptible.

(*d*) Limelos and orangelos are as susceptible as grapefruit, while clemelos, siamelos, satsumelos, and tangelos are not so resistant as the mandarin oranges.

(*e*) The calarins and calashus are as resistant as either parent, while siamors and bigaraldins are susceptible.

(5) All false hybrids are extremely susceptible.

(6) Leaf texture is apparently an important factor in influencing resistance to *Pseudomonas citri* by its host plants. This phase deserves further investigation.

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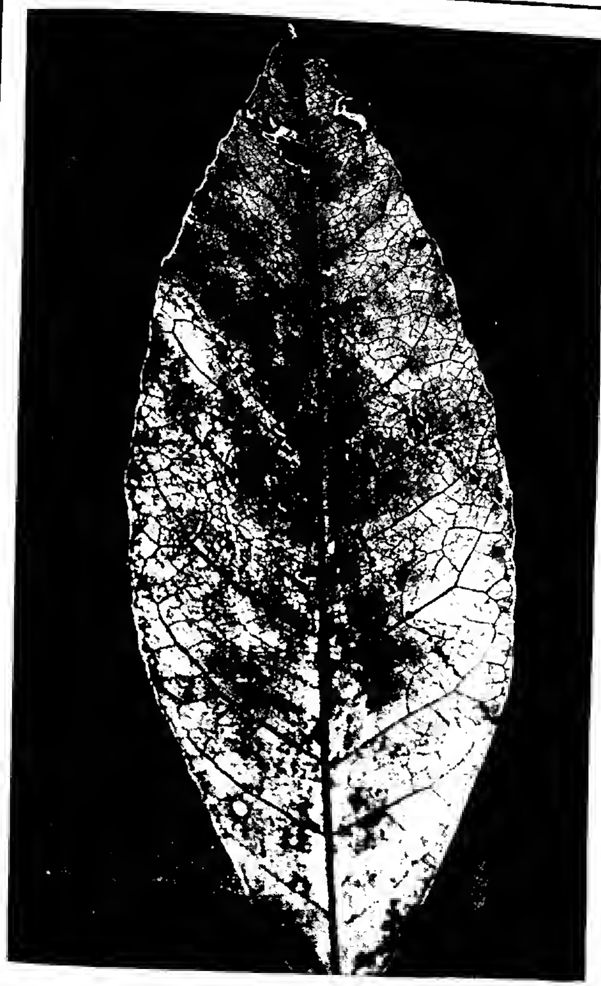
PLATE 57

Leaf of *Casimiroa edulis*, with naturally occurring spots from the greenhouse inoculations. Note that the spots are small, unruptured, and occur only along scratches. Natural size.

PLATE 57



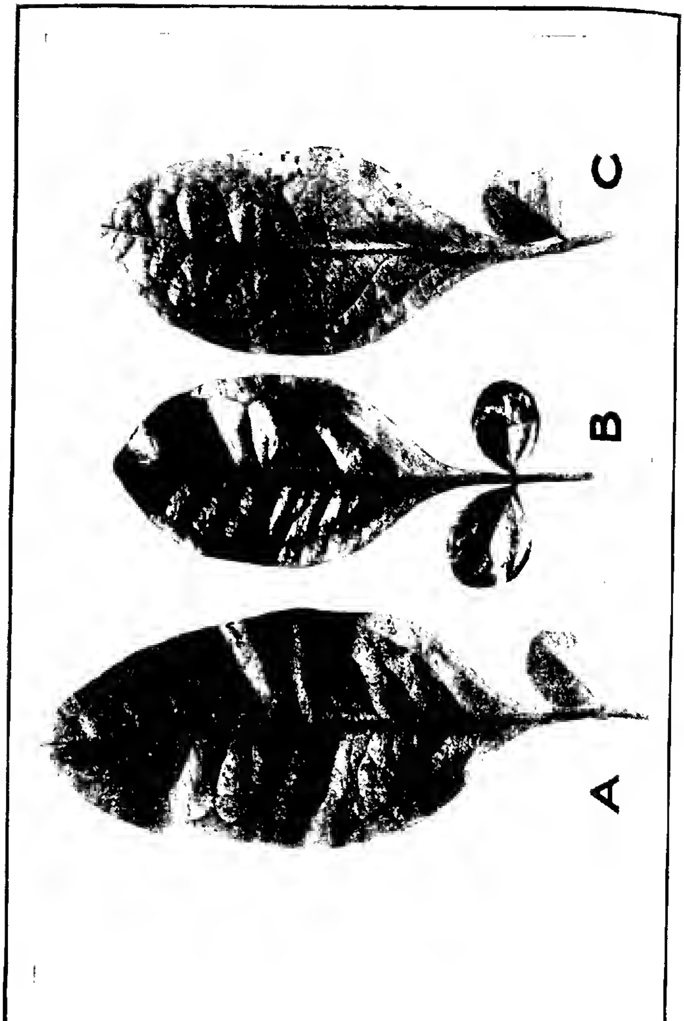

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PLATE 58

Infected leaves from plants of *Chaetospernum glutinosum* in the greenhouse experiments, showing the types of canker spots produced:

A.—Spots extremely small and nontypical.

B.—Spots small, oily, raised, and unruptured.

C.—Spots of medium size, ruptured, and slightly corky.

Natural size.

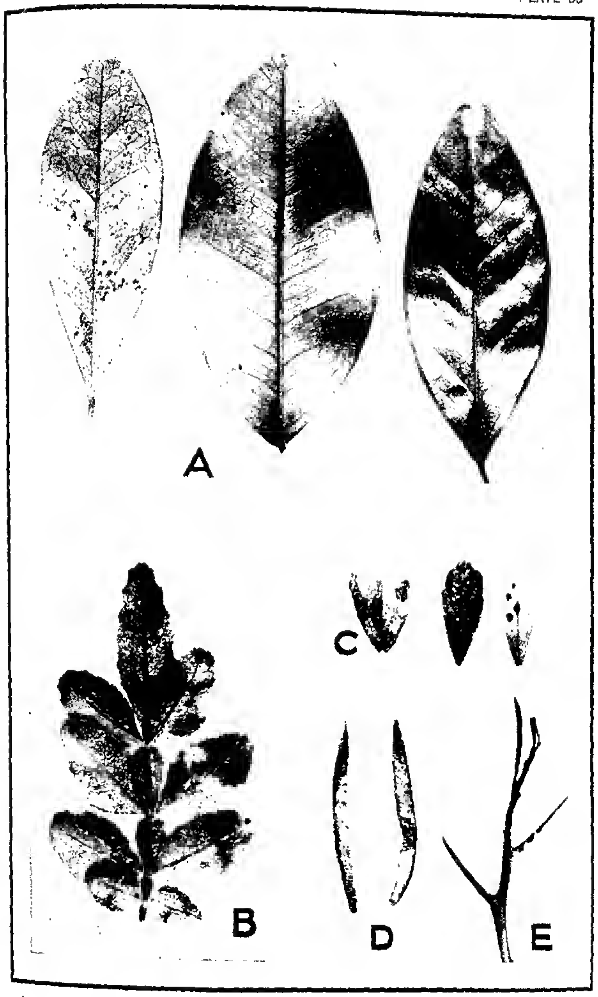
PLATE 59

A.—Leaves of *Atalantia citrioides* and *A. ceylonica* (center) from plants in the greenhouse experiments, showing the canker spots typically produced on these plants. Natural size.

B.—Compound leaf of *Hesperthusa crenulata* from isolation field, with naturally occurring canker spots on two of the leaves. Natural size.

C.—Leaves of *Microcitrus Garrowayi* from plants in the greenhouse experiments, with different types of canker spots. These spots are characteristic of all found on *Microcitrus* spp. × 2.

D, E.—Infected leaves from twigs of *Eremocitrus glauca* from greenhouse plants, showing the large flat spots on the leaves and the rather corky spots on the twig and thorn. × 3.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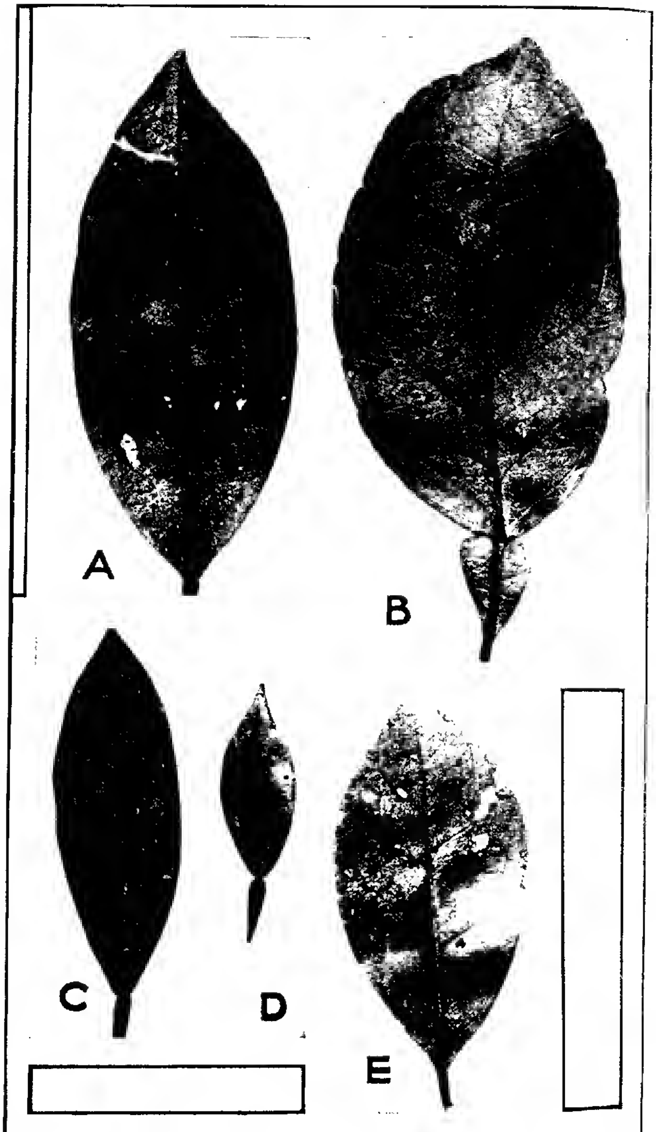
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PLATE 60

A.—Typically infected leaf of *Fortunella margarita.*

B.—Old leaf of *Citrus grandis*, with raised, compact, oily, unruptured spots. Compare leaf texture and type of spots with those of *Fortunella margarita.*

C.—*Fortunella Hindsii*, with ruptured corky spots. Compare with the spots found on *F. margarita.*

D.—*Citrus* sp, Kansu or Yuzu orange. Small spots produced on the leaves of these plants are numerous but never increase in size.

E.—*Citrus aurantifolia*, showing typical infections. All natural size and from greenhouse experiments.

PLATE 61

Upper and lower leaf surfaces of a *Citrus hystrix* leaf with a heavy natural canker infection. The yellow zone around the spots is very distinctly shown. Natural size.

PLATE 62

Typically infected leaves of *Citrus grandis* from field plants showing extreme susceptibility. Note number, size, and corkiness of spots, also the rather large zone surrounding the spots. $\times \frac{2}{3}$.

PLATE 63

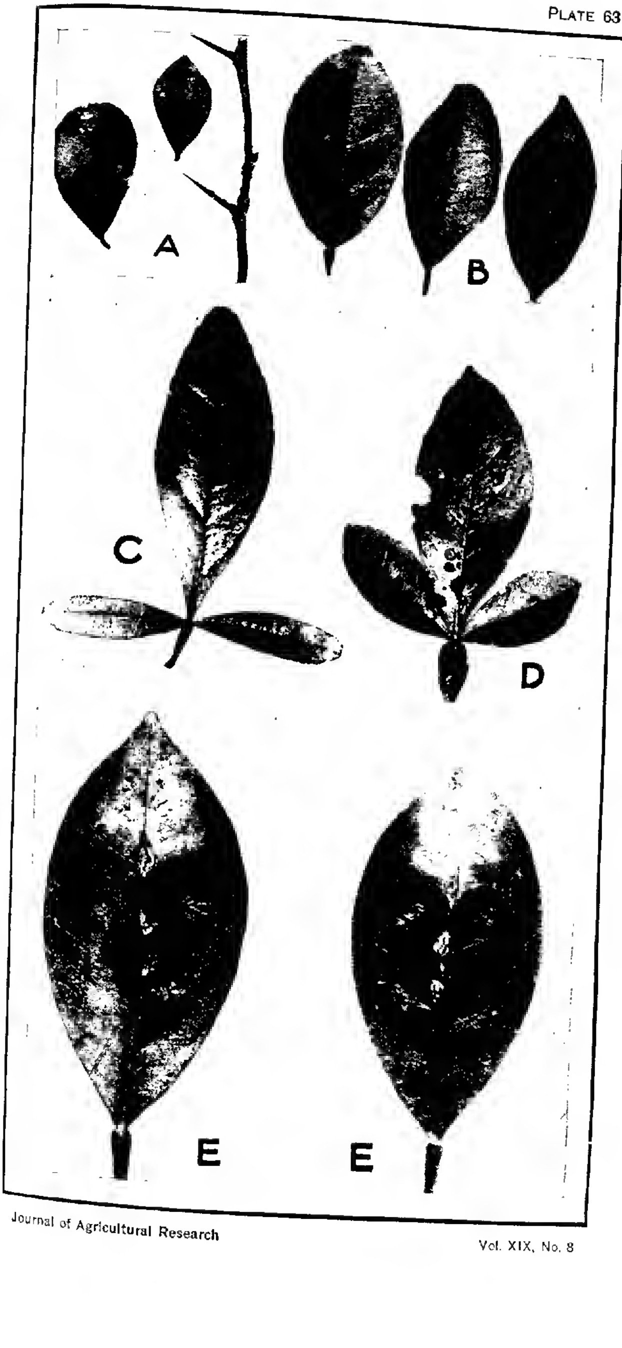
A.—Leaves and twigs of faustrime from greenhouse experiment with typical spots. Compare with those on *Microcitrus australis.*

B.—Types of spots found on *Citrus nobilis* (King of Siam, Naranjita, and tangerine). × ½.

C.—Leaf of the citrangequat from greenhouse experiment. The spots here are extremely small and never increase in size. This is one of the few leaves that have been successfully inoculated.

D.—Citrumelo leaf with typical canker spots. All hybrids of *Poncirus trifoliata* have the same type of spot.

E.—Upper and lower surface of a naturally infected leaf of *Citrus mitis* in the field. Practically all spots occur along the midrib.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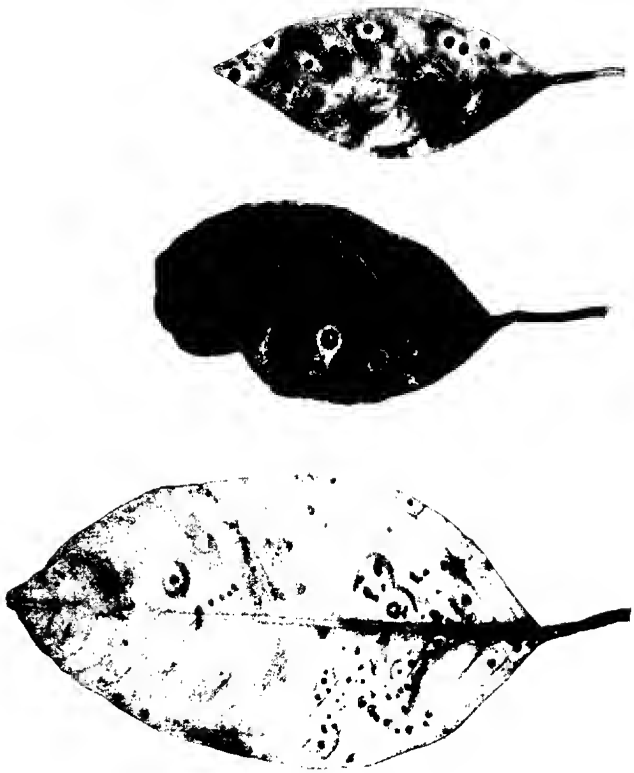

PLATE 64

Naturally infected leaves of *Citrus nobilis* var. *unshiu* from the field, showing various types of spots produced. As a rule the spots on the leaf to the left are found most frequently. All leaves represent rather severe infections. × ¾.

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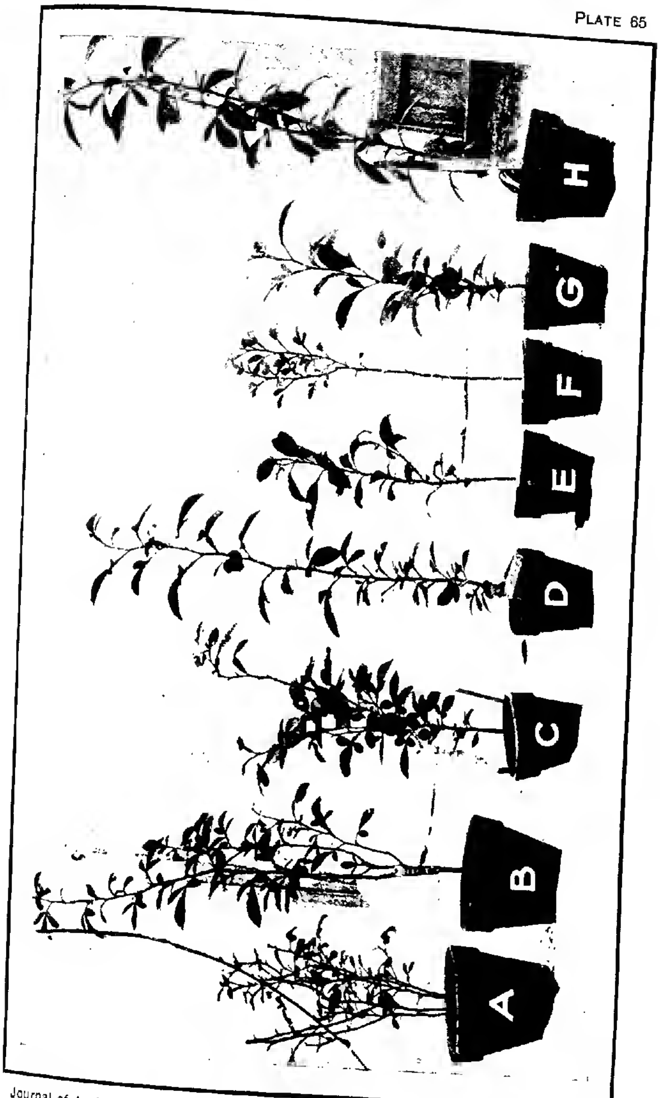
PLATE 65

Some of the hybrids of *Poncirus trifoliata*, showing vigor, type of growth, leaf characters, and relative susceptibility to citrus-canker, arranged in order of their susceptibility:

A.—*P. trifoliata*.
B.—Rusk citrange.
C.—Citrumelo.
D.—Citradia.
E.—Citrandarin.
F.—Cicitrange.
G.—Citranguma.
H.—Citrangequat.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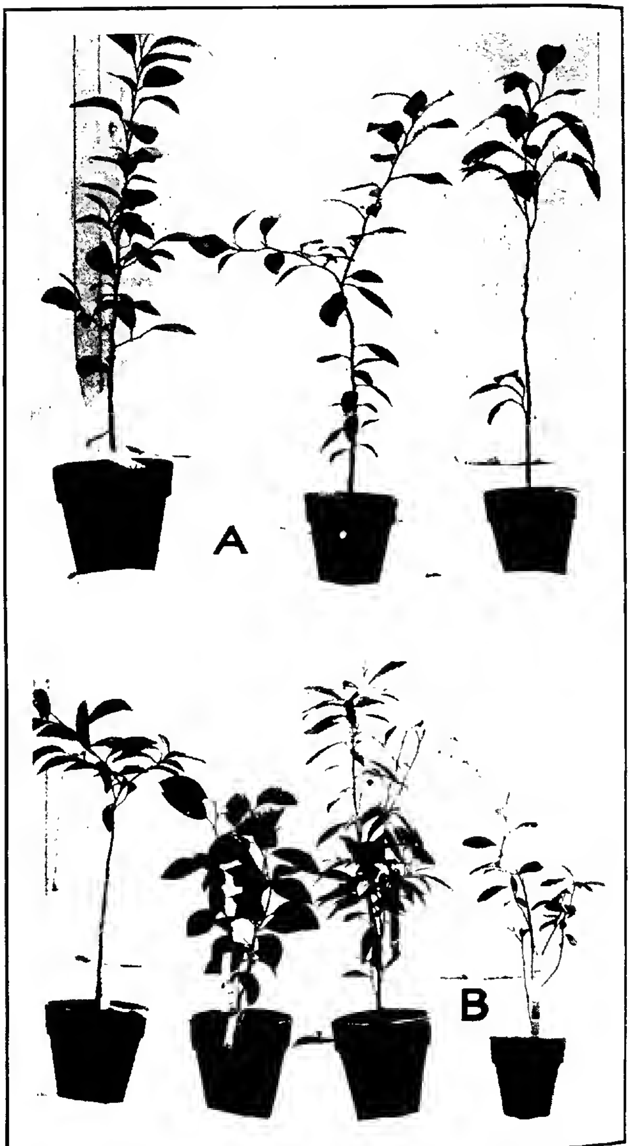
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PLATE 66

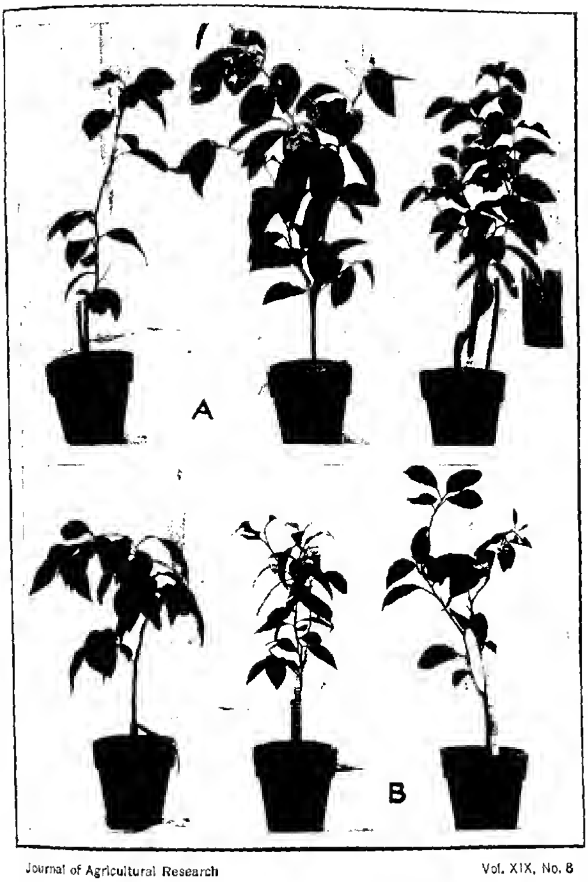
A.—Limelos in the greenhouse inoculation experiments, showing type of growth, leaf characters, and susceptibility to citrus-canker.

B.—Limequats in the greenhouse inoculation experiments, showing type of growth, character of leaves, and susceptibility to citrus-canker. The large, broad leaf forms at the left are more susceptible than the narrower leaf forms at the right.

PLATE 67

A.—Siamelos in the greenhouse inoculation experiments, showing type of growth, leaf characters, and susceptibility to citrus-canker.

B.—Comparison of type of growth, leaf characters, and susceptibility to citrus-canker in clemelo, satsumelo, and tangelo in the greenhouse inoculation experiments.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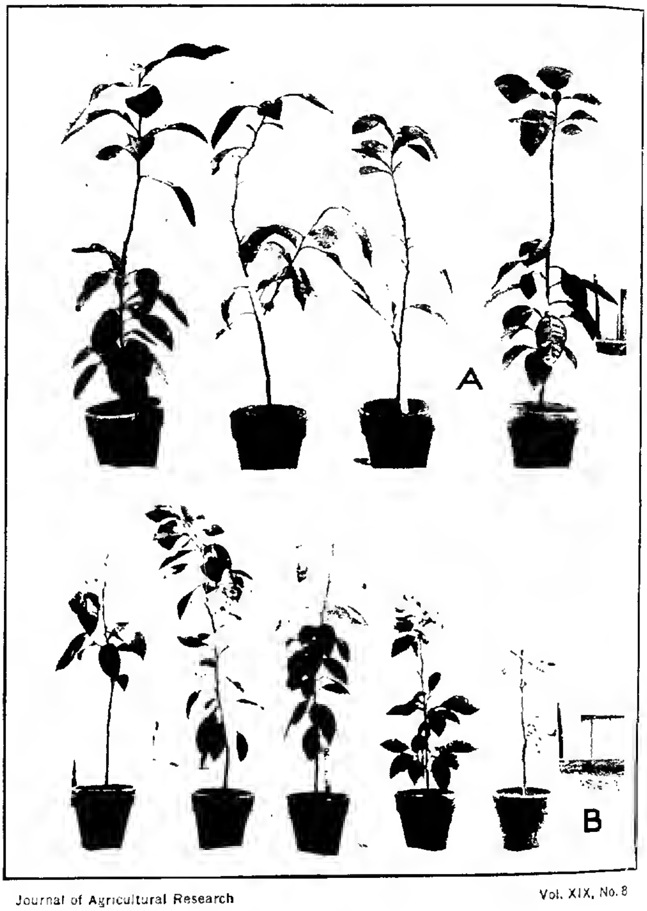
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PLATE 68

A.—Results of the greenhouse inoculations with some of the false hybrids. Note defoliation and heavy stem infection.

B.—Tangelos in the greenhouse inoculation experiments, showing type of growth, leaf character, and susceptibility to citrus-canker.

PRESOAK METHOD OF SEED TREATMENT: A MEANS OF PREVENTING SEED INJURY DUE TO CHEMICAL DISINFECTANTS AND OF INCREASING GERMICIDAL EFFICIENCY

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INTRODUCTION

The widespread use of formalin, copper sulphate, and other germicides in seed treatment for the control of seed-transmitted diseases is generally attended by decreased and retarded seed germination. Pathogens on the seed coats, present as dry bacteria, fungus spores, or dormant mycelium, are usually in a resting stage and as such require the use of disinfectants in a fairly strong concentration—1 to 80 for copper sulphate ($CuSO_4$) and 1 to 200 or 1 to 320 for formalin (CH_2O)—which often act very detrimentally on the germinating seed. Even much weaker solutions—1 to 200 for copper sulphate and 1 to 400 for formalin—exhibit retarding and killing effects when used on wheat. The use of lime after copper sulphate, while beneficial to some extent, does not entirely prevent seed injury, nor has the detrimental effect of formalin been so far fully counteracted. The economic importance of the annual loss of grain due to seed treatment is such that during the recent war it occasioned an elaborate series of tests of standard grain disinfectants by the War Emergency Board of the American Phytopathological Society.

In fact (*3*)[2]—

> the difficulty of avoiding injury to the seed from treatment that is too severe or from improper drying after treatment has undoubtedly had more influence in preventing the general spread of the practice of disinfecting seed grain than has the cost of materials or the difficulty of the treatment itself.

In the course of investigations on the blackchaff bacterial disease of wheat (*5*, *6*, *7*, *8*), under the direction of Dr. Erwin F. Smith, a new treatment (*1*) of seed wheat with formalin and copper sulphate has been discovered whereby seed injury due to these disinfectants is either entirely eliminated or is reduced to a negligible minimum, while at the same time the bacteria are rendered more susceptible to the action of the disinfectant. The result has been accomplished by a correlation of two

[1] The author wishes to acknowledge his indebtedness to Dr. Erwin F. Smith for helpful criticism and advice throughout the course of this investigation.

[2] Reference is made by number (italic) to "Literature cited," p. 392.

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fundamental principles of bacteriology and physico-chemistry: First, the established fact that microorganisms in an active vegetative condition or just resuming activity are more susceptible to destructive agents than when in a dry or dormant state; second, the law governing the diffusion of dissolved substances whereby a solvent has a diluting effect on any solute diffusing into it from a stronger solution.

EXPERIMENTAL METHODS

Numerous experiments with the use of dry heat (90° to 110° C. for various periods) for the control of the blackchaff disease had indicated that it was attended either with serious seed injury or else with incomplete control of the bacteria so as to render it unsatisfactory for field use. Exposure to 105° C. for one hour killed all the bacteria, but it also killed a very considerable part of the seeds, while in every case exposure to temperatures between 90° and 100° C. failed to kill all the bacteria. Earlier experiments with mercuric chlorid (1:1,000) and with copper sulphate (1:1,000) begun by Dr. Smith were abandoned because many seeds were killed by the mercuric chlorid and not quite all the bacteria were killed by the copper sulphate. A number of experiments to determine whether the formalin treatment for cereals might also be applicable as a means of controlling the blackchaff disease showed marked injury to the seeds but gave a fairly satisfactory control. The lesser amount of seed injury growing out of long exposures as compared with short exposures is what led to the discovery of the treatment described in the present paper.

Two parallel series of experiments were carried out—one, to determine the effects on the blackchaff organism of various treatments, the other to observe the effects of the same treatments on the germination of wheat. Nine of the most widely grown wheat varieties were used in the latter series: Turkey, Fultz, Marquis, Bluestem, China, Preston, Poole, Fife, and Fulcaster, obtained through the courtesy of the Office of Cereal Investigations.

Treatments for the seed-germination tests were made as follows, except as otherwise stated. Seeds counted out in sets of 100 were placed in loose cheesecloth bags and soaked thoroughly for 10 minutes in the solution to be tested. The surplus liquid was then drained off, and the seeds were placed in covered moist chambers containing several layers of filter paper previously rinsed with the same solution. After definite periods of time the seeds were removed, spread out to dry overnight, and planted the next day in flats or pots in the greenhouse. Untreated seeds were also planted as controls. Later in the course of the work treated seeds were planted outdoors at the Arlington Experimental Farm.

The effect of the various treatments on the blackchaff organism was determined by the following method, devised by Dr. Smith. Wheat seeds in lots of 100 or more were placed in double envelopes of filter paper and sterilized by dry heat at 150° to 160° C. for three hours, in order to kill all internal and surface organisms. After they had cooled, the envelopes were opened with aseptic precautions and the seeds were thoroughly coated with blackchaff bacteria taken from 2- to 4-day-old nutrient agar or potato cultures and used as a heavily clouded bacterial suspension in sterile tubes of tap water. In this way five isolations of the blackchaff organism from as many States were tested. The seeds, after soaking in the bacterial suspension for 20 minutes, were replaced in the envelopes and allowed to dry overnight. By this method each kernel was coated with a dry film of live bacteria such as would occur on badly infected seed under natural conditions. The next day the inoculated and dried seeds were dropped into sterile test tubes containing the disinfectant to be tested. The liquid was drained off after it had acted 10 minutes. The tubes containing the seeds, which now had a thin layer of the solution around each kernel, were placed in moist chambers previously rinsed with the same solution. After definite periods the seeds, still moist, were replaced in the sterile envelopes to dry overnight. The next day they were transferred to nutrient agar previously determined to be suitable for the organism, in poured plates, 10 seeds per plate. Each seed was handled with forceps which had been dipped in alcohol and flamed. Control seeds which had been inoculated but not subsequently treated were also planted on this agar. After at least nine days the final records were made. The controls usually developed a typical blackchaff colony around each kernel. Treated seeds, if all the bacteria thereon were killed by the solution used, remained sterile unless contaminated by other organisms or slowly produced blackchaff colonies if the disinfectant had not been fully effective. This method is a good index of the effect on the bacteria of the various treatments studied. The seeds are sterilized by dry heat externally and internally without leaving any antiseptic residue such as might be left by chemical sterilization. The treatment under consideration is performed upon dried but live bacteria which are found on the seed coat exactly as they would occur in field practice, except that ordinarily they would be less viable and there would be fewer of them. The subsequent exposure on agar plates to optimum conditions for bacterial growth reveals the effect of the treatment, in that the bacteria on the seeds, if uninjured by the treatment, are enabled to develop characteristic colonies, the slowness of their development being a very good index of the proportion killed. Over 5,500 seeds were treated in this manner in the course of the investigation.

EXPERIMENTAL STUDIES

EFFECT OF FORMALIN TREATMENT ON BACTERIUM TRANSLUCENS VAR. UNDULOSUM SMITH, JONES, AND REDDY

As the bacterial blackchaff disease has often been found together with the covered smut of wheat in western fields, experiments were first performed to determine whether the formalin treatment for smut would at the same time control the blackchaff disease. Following the procedure above outlined, sterilized wheat seeds were inoculated with virulent isolations of the blackchaff organism and then treated for various periods with formalin 1 to 200 and 1 to 400 (1 part of 36.6 per cent formalin to 200 or 400 parts sterile tap water) and finally dried and planted on agar plates. The results of treatments of over 3,000 wheat seeds in four experiments are summarized in Table I.

TABLE I.—*Effect of formalin treatment on blackchaff bacteria on wheat seeds*

Treatment.	Total number of seeds used.	Percentage developing typical blackchaff colonies.	Percentage remaining sterile.	Percentage contaminated with fungi or bacteria other than blackchaff.
Formalin 1:200 overnight	294	0.0	82.3	17.7
Formalin 1:400 for 3 hours	1,000	.1	92.6	7.3
Formalin 1:400 for 6 hours	1,000	.0	96.7	3.3
Formalin 1:400 for 12 hours	597	.0	84.7	15.3
Controls inoculated but not treated	320	82.6	16.2	1.2

The data show that the blackchaff bacteria, dried on wheat seeds as under natural conditions, can be held under control by the formalin 1 to 400 treatment, especially when exposed for six hours or longer.

Since all the formalin used in treating the seeds had evaporated during the overnight drying, no residual solution could have been left on the seeds in the plates to prevent bacterial growth. Neither did 24 to 48 hours' drying after inoculation kill the organisms, as was shown by growth in the controls, which acted as an index of the viability of the dried organisms as well as of the suitability for bacterial growth on the part of the particular lot of media used. The conclusion is evident that absence of blackchaff bacterial growth around treated seeds was due only to the effect of the treatment.

GREENHOUSE EXPERIMENTS WITH FORMALIN AND COPPER SULPHATE

EFFECT OF FORMALIN TREATMENT ON GERMINATION OF WHEAT SEED

Parallel with the experiments made to determine the effect on the bacteria, a series was carried out to determine the effect of the two formalin solutions, as used above, on the germination of wheat seed. The 1 to 200 strength formalin was not tried after the second test because

it appeared too injurious to germination to be of any practical value in the field. A total of 6,300 seeds of three varieties was treated in this series and planted in flats in the greenhouse. Records of germination, counting all seedlings above ground on the seventh day after planting for experiment I and those above ground on the ninth day for experiments II and III, are given in Table II.

TABLE II.—*Effect of formalin treatment on germination of wheat seed*

Treatment.	China.				Bluestem.				Turkey.			
	Average percentage of germination.				Average percentage of germination.				Average percentage of germination.			
	Exp. I.	Exp. II.	Exp. III.	Average.	Exp. I.	Exp. II.	Exp. III.	Average.	Exp. I.	Exp. II.	Exp. III.	Average.
Control	78	63	63	68	50	53	43	49	56	63	75	65
Formalin 1:400 for 3 hours	56	48	40	48	24	26	31	27	42	45	65	51
Formalin 1:400 for 6 hours	53	40	45	46	25	34	35	31	48	53	62	54
Formalin 1:400 for 12 hours	74	53	46	58	39	33	40	37	50	54	68	57
Formalin 1:200 for 3 hours	39	15	....	27	9	19	....	14	23	31	....	27
Formalin 1:200 for 6 hours	37	33	....	35	13	17	....	15	17	32	....	25
Formalin 1:200 for 12 hours	57	29	....	43	9	16	....	13	34	36	....	35

The preceding table shows grave injury to germination where the stronger solution was used. There was also marked retardation of germination. Formalin 1 to 400, while much less harmful, caused an appreciable decrease in germination as compared with the controls. It was observed, however, that the 12-hour treatment (1: 400) invariably produced less retardation and loss in germination than the 3-hour treatment. This was repeatedly evident for each variety (Pl. 69). A fourth test for Turkey wheat (part of experiment IV), using 400 seeds treated with formalin 1 to 400 for 1 hour, 400 seeds treated 12 hours, and 100 seeds untreated, gave on the sixth day after planting 45 per cent germination for the 1-hour treatment, 59 per cent for the 12-hour treatment, and 62 per cent for the controls. These results, so contrary to what might have been expected, led to the experiments to be described. A search of the literature after these results had been obtained disclosed a similar condition in a number of cases not commented on by the authors—that is, less injury from long exposures than from short ones.

Such a condition is found in an analysis which I have made of the data presented by a subcommittee of the War Emergency Board of Plant Pathologists (*4*) on the effect of formalin 1 to 320 acting for various periods on different cereal seeds. With wheat, in 18 tests out of 25

the short 2-hour treatment shows more injury than a longer treatment; with barley, 14 out of 19 tests show more injury from the short treatment; and so with oats in 29 out of 41 tests and with rye in 2 out of 3 tests. In some cases there is a steady decrease of injury as the treatment period lengthens.

A study of the tables given by Stuart (9) in his paper on the effect of formalin on oat germination shows almost invariably greater injury to germination caused by 2-hour treatment than by 4-hour treatment of seeds, and a similar effect on the final yield of grain and straw—a fact not commented on by that writer.

These facts led to the conjecture that the formaldehyde content in the seeds at the end of 3 hours was really stronger than in the 12-hour treated seeds. Such a condition might be explained by the hypothesis that the dry seeds absorb the formaldehyde itself more rapidly than they do the water and that by diffusion later this is diluted to a strength more like the original solution through continued absorption of water by the cell walls and cells.

Theoretically, therefore, if the 3-hour treatment could be made in such a way that the final solution content of the seeds would be comparable in amount and dilution with that finally present in 12-hour treated seeds, the effects on germination should also be similar. By impregnating the cell walls and cells of dry seeds with water and then treating with formalin for three hours, it appeared as if this result might be attained; for, in accordance with the laws of diffusion of dissolved substances, the formalin should be diluted as it diffused into the water-saturated seed tissues.

Acting on this hypothesis, wheat seeds were first soaked in tap water for 10 minutes, drained, and kept moist for 9 hours, then soaked thoroughly in formalin 1 to 400 for 10 minutes, drained, and kept moist for 3 hours in order that the water absorbed during the first 9 hours might weaken the full strength solution diffusing into the cells during the next 3 hours and might result in less injury than was caused by the 3-hour treatment of dry seeds. Following this, numerous experiments were made, varying the length of time during which the seeds were kept moist but starting out always with a preliminary short plunge into water. This method—short exposure under water followed by varying lengths of exposure to moist air (covered)—has been designated throughout this paper as the "presoak" method of seed treatment. In the same way, whenever exposure to formalin is mentioned it means always that the seeds were plunged into the formalin water solution for a short period only (usually 10 minutes) and then kept moist (covered) for the designated number of hours.

The seeds of two varieties so treated were planted in the greenhouse with 12-hour treated seeds and controls. Table III records the results observed.

TABLE III.—*Effect of 9 hours' presoaking followed by formalin 1 : 400 treatment*

Treatment.	Percentage of germination.					
	China.	Bluestem.	China.			Marquis, Exp. VI.
			Exp. V.	Exp. VI.	Average.	
Control	70	78	46	50	48	59
Formalin 1:400 for 10 minutes, covered 12 hours	60	60	40	43	42	56
Formalin 1:400 for 10 minutes, covered 3 hours	......	......	35	23	29	49
Water for 10 minutes, covered 9 hours, formalin 1:400 for 10 minutes, covered 3 hours	67	72	45	42	44	58

Table III shows for the presoaked seeds not only a decrease in injury over the 3-hour treated seeds as previously observed but also a decided increase in germination over the seeds treated 12 hours with formalin. The latter show a loss of 10 to 18 per cent, the former a loss of only 3 to 6 per cent. At the same time the presoaked seeds produced larger and more vigorous plants than the controls.

Very clearly the presoak treatment followed by 3 hours of formalin treatment showed a distinct advantage over the usual formalin treatment. As this appeared to be a promising method of reducing formalin injury, a thorough test was then made with all the varieties of wheat at hand. It appeared possible that for some varieties a 9-hour presoak might begin germination before treatment, thus rendering the seeds very susceptible to injury, whereas, a formalin-treatment period longer than 3 hours appeared desirable for control purposes. For these reasons, the presoak period was reduced to 6 hours, followed by a 6-hour treatment with formalin.

EFFECT OF 6 HOURS' PRESOAKING FOLLOWED BY FORMALIN 1 TO 400 TREATMENT ON GERMINATION OF WHEAT SEED

The presoak treatment in every instance was given by soaking the seeds 10 minutes in water, then draining off the surplus water and keeping the seeds in moist chambers 6 hours, allowing the seeds to absorb the surface moisture film during this period. After shaking off all possible surface water, the next step was to soak the seeds thoroughly in a 1 to 400 formalin solution, stirring and rinsing up and down to bring the solution in contact with each kernel. After 10 minutes in this solution the seeds were removed, drained, and kept moist for 6 hours in moist chambers previously rinsed with the same solution. After treatment the seeds were dried overnight and planted in the greenhouse, 100 seeds per pot, in duplicate or triplicate.

FIG. 1.—Graph showing effect of formalin 1 to 400 treatments with and without presoaking: A, control, untreated; B, seeds soaked in formalin 1 to 400 for 10 minutes, drained, and kept moist (covered) 12 hours, dried overnight, and planted; C, seeds soaked in water 10 minutes, drained, and kept moist (covered) 6 hours, soaked in formalin 1 to 400 for 10 minutes, drained, and kept moist (covered) 6 hours, dried overnight, and planted. Records of germination were taken on the sixth day after planting in the greenhouse.

Using a 6-hour presoak followed immediately by a 6-hour treatment with formalin 1 to 400, 6 experiments were carried on in the greenhouse with 23,700 seeds of nine varieties, the test for each variety being repeated several times. Table IV and figure 1 show the data obtained on the sixth day after planting in each test, this date being chosen as showing not only the relative percentage of germination but particularly revealing any retardation or acceleration. At the same time, the effect of this method on the blackchaff bacteria was determined, as discussed later.

TABLE IV.—*Effect of 6 hours' presoaking followed by formalin 1 : 400 treatment on germination of wheat seed*

Treatment.	Average percentage of germination.											
	Fife.				Bluestem.				Preston.			
	Exp. V.	Exp. VI.	Exp. VII.	Average.	Exp. V.	Exp. VI.	Exp. VII.	Average.	Exp. V.	Exp. VI.	Exp. VII.	Average.
Control	89	74	75	79	83	79	82	81	79	75	77	77
Formalin 1:400 for 10 minutes, covered 12 hours	75	66	75	72	61	54	66	60	53	51	63	56
Water for 10 minutes, covered 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	77	71	86	78	74	64	82	73	66	64	82	71

Treatment.	Average percentage of germination.								
	Marquis.				China.			Turkey.		
	Exp. V.	Exp. VI.	Exp. VII.	Average.	Exp. V.	Exp. VI.	Average.	Exp. V.	Exp. VI.	Average.
Control	82	79	84	82	52	38	45	60	61	61
Formalin 1:400 for 10 minutes, covered 12 hours	54	62	70	62	44	34	39	60	52	56
Water for 10 minutes, covered 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	61	75	82	73	50	44	47	71	50	61

Treatment.	Average percentage of germination.							
	Poole.				Fultz.			
	Exp. VIII.	Exp. IX.	Exp. X.	Average.	Exp. VIII.	Exp. IX.	Exp. X.	Average.
Control	72	80	92	81	55	83	74	71
Formalin 1:400 for 10 minutes, covered 6 hours	67	85	70	74	57	70	57	61
Formalin 1:400 for 10 minutes, covered 12 hours	65	82	79	75	42	66	62	57
Water for 10 minutes, covered 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	76	89	89	88	60	78	76	71

TABLE IV.—*Effect of 6 hours' presoaking followed by formalin 1 : 400 treatment on germination of wheat seed*—Continued

Treatment.	Fulcaster.				Turkey.			
	Exp. VIII.	Exp. IX.	Exp. X.	Average.	Exp. VIII.	Exp. IX.	Exp. X.	Average.
Control	82	84	79	82	41	74	64	59
Formalin 1 : 400 for 10 minutes, covered 6 hours	62	67	60	63	[a]56	64	48	56
Formalin 1 : 400 for 10 minutes, covered 12 hours	68	72	69	70	35	67	61	54
Water for 10 minutes, covered 6 hours, formalin 1 : 400 for 10 minutes, covered 6 hours	77	80	83	80	46	71	65	57

[a] Result from one pot only, the other having been overturned.

For each variety of wheat used the result is the same—a marked decrease in retardation and injury to germination where the presoak method

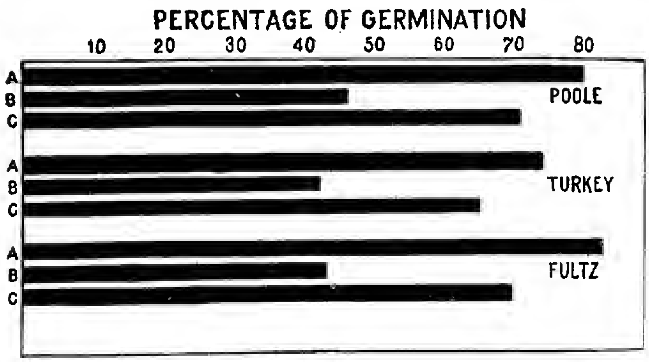

FIG. 2.—Graph showing effect of formalin 1 to 200 treatments with and without presoaking: A, control, untreated; B, seeds soaked in formalin 1 to 200 for 10 minutes, drained, and kept moist (covered) 6 hours, dried overnight, and planted; C, seeds soaked in water 10 minutes, drained, and kept moist (covered) 6 hours, then soaked in formalin 1 to 200 for 10 minutes, drained, and kept moist (covered) 6 hours, dried overnight, and planted. Records of germination were taken on the sixth day after planting in the greenhouse.

of treatment was used, as compared with the 6- or 12-hour formalin treatment without presoaking. In the case of the three varieties most susceptible to formalin—Bluestem, Preston, and Marquis—germination of the presoak-treated seeds is within 6 to 9 per cent of the controls, while there is a reduction of 20 to 21 per cent in the seeds treated without presoaking. The other six varieties show practically all injury eliminated by the presoak treatment. The relative appearance of controls, treated plants, and presoak-treated plants on the sixth day is shown in Plates 70 and 71. A very noticeable stimulation in vigor was observed in

all of the presoak-treated seeds as compared with the controls. This is brought out also in Plate 72.

EFFECT OF 6 HOURS' PRESOAKING FOLLOWED BY FORMALIN 1 TO 200 TREATMENT ON GERMINATION OF WHEAT SEED

The beneficial effect of the presoak method is strikingly shown in an experiment where a much stronger solution of formalin was used. Seeds of three varieties were treated for 10 minutes with formalin 1 to 200 and were then kept moist (covered) for 6 hours, dried overnight, and planted in the greenhouse. Another set of seeds received the same treatment but were first soaked in water 10 minutes, drained, and kept moist 6 hours before receiving the formalin treatment. This strength of 1 to 200 had previously been found to cause a very considerable injury to germination. Table V and figure 2 show the percentage of germination on the sixth day.

Table V.—*Effect of 6 hours' presoaking followed by formalin 1:200 treatment on germination of wheat seed*

Treatment.	Average percentage of germination.		
	Poole.	Turkey.	Fultz.
Control	80	74	83
Formalin 1:200 for 6 hours	46	42	43
Water for 6 hours, formalin 1:200 for 6 hours	71	65	70

Here the 6-hour formalin 1 to 200 treatment reduced germination 32 to 40 per cent below that of the controls, while the reduction was only 9 to 13 per cent where the same treatment was preceded by 6 hours' water presoak (Pl. 73.)

EFFECT OF 6 HOURS' PRESOAKING FOLLOWED BY FORMALIN 1 TO 320 TREATMENT ON GERMINATION OF WHEAT SEED

Formalin 1 to 320, or one pound of formalin to 40 gallons of water, was next used, since that is the strength now recommended for the cereal smuts. Besides the presoak procedure so far followed, a test was made (experiment XI) of the effect of an actual soaking in water for 5 hours, followed by thorough draining for a few minutes, then 10 minutes' soaking in the formalin 1 to 320, then covering for 7 hours before drying and planting. Experiment XII was conducted to test the effect of an actual soaking in water for 4 hours, followed by thorough draining for a few minutes, then soaking in formalin 1 to 320 for 10 minutes, draining, covering 6 hours, drying, and planting. The results obtained are shown in Table VI and figure 3.

TABLE VI.—*Effect of 6 hours' presoaking followed by formalin 1 : 320 treatment on germination of wheat seed under greenhouse conditions*

Treatment.	Average percentage of germination.					
	Poole.			Fife.		
	Exp. XI.	Exp. XII.	Average.	Exp. XI.	Exp. XII.	Average.
Control	94	85	90	79	83	81
Formalin 1 : 320 for 10 minutes, drained, covered 6 hours	66	55	61	59	62	61
Formalin 1 : 320 for 10 minutes, drained, covered 12 hours	67	71	69	58	64	61
Water for 10 minutes, covered 6 hours, formalin 1 : 320 for 10 minutes, drained, covered 6 hours	90	88	89	80	90	85
Actual soaking in water 5 hours, formalin 1 : 320 for 10 minutes, covered 7 hours	88	.....	88	77	.....	77
Actual soaking in water 4 hours, formalin 1 : 320 for 10 minutes, covered 6 hours	.....	79	79	.....	85	85

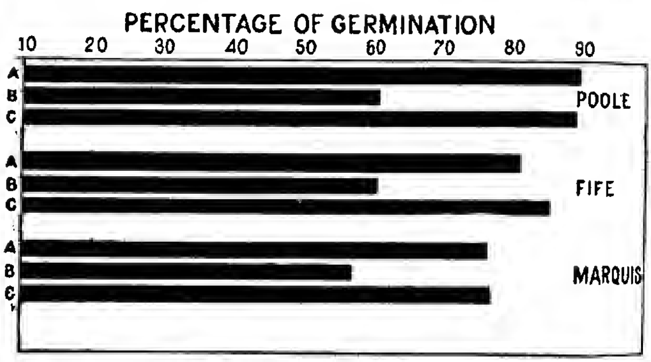

FIG. 3.—Graph showing effect of formalin 1 to 320 with and without presoaking: A, control, untreated; B, seeds soaked in formalin 1 to 320 for 10 minutes, drained, kept moist (covered) 6 hours, dried overnight, and planted; C, seeds soaked in water 10 minutes, drained, kept moist (covered) 6 hours, soaked in formalin 1 to 320 for 10 minutes, drained, kept moist (covered) 6 hours, dried overnight, and planted. Records of germination were taken on the sixth day after planting in the greenhouse.

A marked retardation and diminished germination is shown by both formalin treatments without presoaking. The 6-hour formalin treatment preceded by 6 hours' presoaking yielded plants similar to the controls in percentage of germination, and they were very evidently stimulated, as shown in Plates 74, 75, and 76. Actual soaking in water did not appear to be so beneficial to the vigor of the seedlings as the procedure of merely keeping the seeds moist for 6 hours before treating with formalin. (See Pl. 75, fig. 3.)

EFFECT OF PRESOAKING WHEN USED WITH COPPER SULPHATE FOR WHEAT AND BARLEY SEED

The striking reduction in formalin injury to seed germination when the presoak method was used led to trials of this method in conjunction with copper sulphate. Six hundred wheat seeds of Fife and Fulcaster varieties were soaked in a very strong copper-sulphate solution (1:80, or 1 pound to 10 gallons of water) for 10 minutes, drained 20 minutes, dipped for a moment in milk of lime, and dried. A like number of seeds received the same treatment except that they were kept moist for 8 hours after

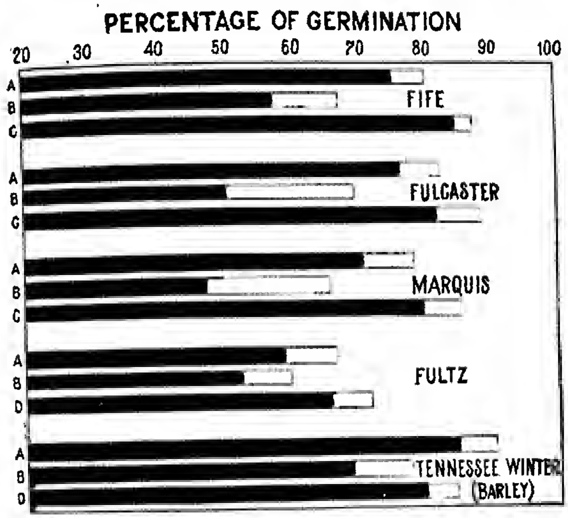

FIG. 4.—Graph showing effect of copper sulphate 1 to 80, with and without presoaking, on wheat and barley seed germination: A, control, untreated; B, seeds soaked in copper sulphate 1 to 80 for 10 minutes, drained, kept moist 20 minutes, then limed, dried overnight, and planted; C, seeds first soaked in water 10 minutes, drained, and kept moist (covered) for 8 hours, then treated as in B; D, seeds first soaked in water 10 minutes, drained, kept moist (covered) for 6 hours, then limed, dried overnight and planted. Records of germination were taken on the fifth and seventh days after planting in the greenhouse.

soaking 10 minutes in tap water. Four hundred seeds were used as controls. All seeds were planted in the greenhouse after drying overnight. The experiment was later repeated, using barley also and wheat seeds of Fultz and Marquis varieties, kept moist in the manner described for 6 hours before treatment. The photographs (Pl. 76, 77) and figure 4 show the results obtained.

In these experiments the injury produced by the copper-sulphate treatment was prevented by the use of the 6- or 8-hour presoak. The 6-hour presoak appears preferable, because a longer period, by starting

seed germination, may render the seed unusually susceptible to the subsequent copper-sulphate treatment and thus defeat its purpose.

A marked increase in the percentage of germination was observed in the presoak-treated seeds over the controls. This was probably due not only to the lack of injury in the former but to the residual effect of the copper sulphate and lime, which, by preventing seed infection through soil organisms, enabled more seeds to germinate. There was also a marked stimulating effect on the growth of the seedlings.

The use of the presoak method of treatment also reduces copper-sulphate injury in barley, as shown in Plate 76, figure 3. The fact that the presoak method can reduce seed injury from formalin and copper sulphate, two disinfectants of widely different chemical nature, suggests the possibility of its use in conjunction with mercuric chlorid also, another commonly used seed disinfectant.

FIELD EXPERIMENTS WITH FORMALIN AND COPPER SULPHATE

EFFECT OF 6 HOURS' PRESOAKING FOLLOWED BY FORMALIN 1 TO 320 TREATMENT ON GERMINATION OF WHEAT SEED UNDER FIELD CONDITIONS

That formalin injury to germination can be greatly decreased under field conditions when the presoak method is used is shown by the following experiment. Using seven wheat varieties, 16,800 seeds were planted on a uniform level plot at the Arlington Experimental Farm. For each variety 12 rows of 200 seeds each were distributed as follows: four rows of controls, four rows of seeds treated with formalin 1 to 320 for 6 hours, and four rows of seeds similarly treated but presoaked 6 hours. The results were striking. In each variety the central four rows, which received the usual treatment recommended for smut, showed a marked decrease in germination (27 to 53 per cent, averaging 38 per cent for the seven plots) while in each case the four rows receiving the presoak formalin treatment scarcely differed in appearance from the controls (Pl. 78). Table VII and figure 5 give the data obtained.

TABLE VII.—*Effect of 6 hours' presoaking followed by formalin 1:320 treatment on germination of wheat seed under field conditions*

Treatment.	Average percentage of germination.													
	Fulcaster		Fife.		Turkey.		Poole.		China.		Bluestem		Marquis	
	10th day.	20th day.	10th day.	20th day	10th day.	20th day.	10th day	20th day.	10th day.	20th day.	10th day.	20th day	10th day	20th day.
Control	60.0	66.3	58.4	59.6	40.6	50.4	60.2	64.3	26.1	32.6	62.2	63.0	50.2	53.9
Formalin 1:320 for 10 minutes, covered 6 hours	32.1	36.8	31.0	41.8	24.2	28.6	20.4	29.8	14.5	21.1	32.5	44.5	24.2	39.1
Presoaked in water, 10 minutes, kept moist 6 hours, formalin 1:320 for 10 minutes, covered 6 hours	52.5	56.4	56.2	58.3	40.8	51.2	53.5	58.3	30.8	35.4	43.6	53.6	46.8	51.6

The results obtained in the field fully corroborate the greenhouse experiments as to the beneficial effect of the presoak method and show that in actual field practice wheat seed injury caused by the formalin treatment recommended for covered smut can be practically eliminated by allowing the seeds to absorb water in the manner prescribed for six hours

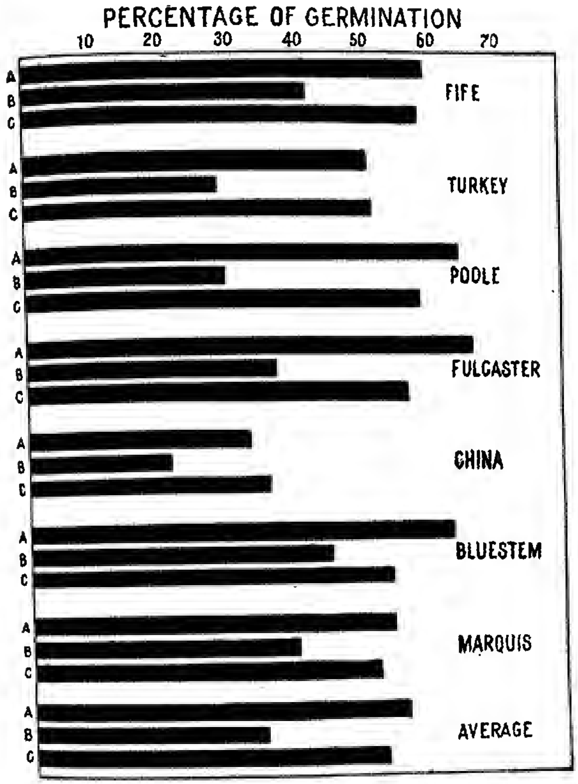

FIG. 5.—Graph showing effect of formalin 1 to 320, with and without presoaking, on wheat seed germination under field conditions: A, control, untreated; B, seeds soaked in formalin 1 to 320 for 10 minutes, drained, kept moist (covered) 6 hours, dried overnight, and planted; C, seeds soaked in water 10 minutes, drained, kept moist (covered) 6 hours, soaked in formalin 1 to 320 for 10 minutes, drained, kept moist (covered) 6 hours, dried overnight, and planted.

before receiving this treatment. The effect of the presoak method of treatment in also eliminating retardation of germination is an important factor in preventing the attack of soil fungi on seeds or very young seedlings unduly delayed in germination. Plate 78 shows the appearance of the field plots on the sixteenth day after planting.

EFFECT OF SIX HOURS' PRESOAKING FOLLOWED BY FORMALIN AND COPPER-SULPHATE TREATMENTS ON HALF-BUSHEL LOTS OF WHEAT SEED

The next series of experiments was made to determine (1) the effect of the presoak method of treatment, using wheat seed in half-bushel lots

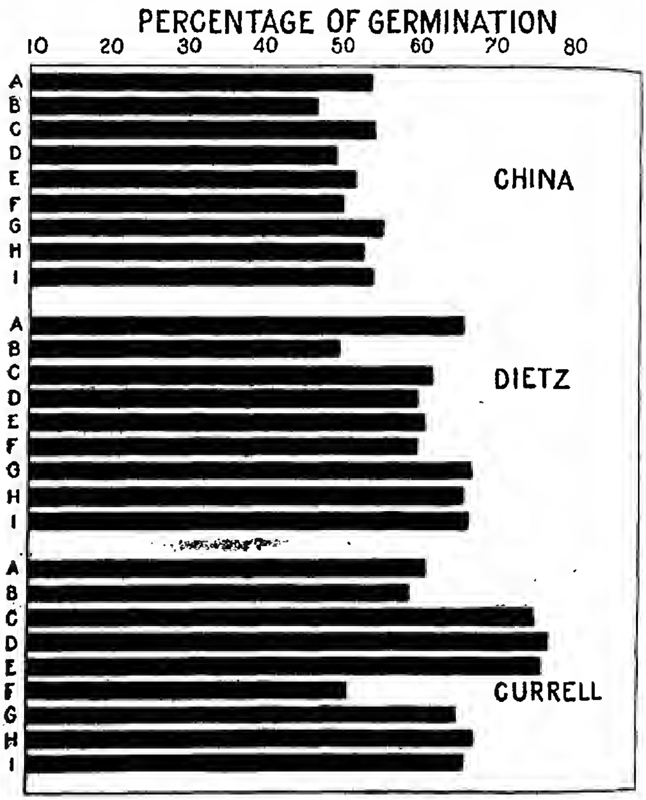

FIG. 6.—Graph showing effect of formalin and copper-sulphate presoak treatments of ½-bushel wheat seed lots: A, control, untreated; B, seeds soaked in formalin 1 to 320 for 10 minutes, drained, covered 6 hours, dried, and planted; C, seeds soaked in water 10 minutes, drained, covered 6 hours, then treated with formalin as in B, and seeds from upper one-fourth planted; D, seeds from central part of same lot as C; E, average germination of C and D; F, seeds soaked in copper sulphate 1 to 80 for ½ hour, followed by milk of lime, and dried; G, seeds soaked in water 10 minutes, drained, covered 6 hours, then treated with copper sulphate as in F, and seeds from upper one-fourth planted; H, seeds from central part of same lot as G; I, average germination of G and H. In the Currell variety the same procedure was used except that the seeds were sprinkled instead of soaked.

as in practical usage; (2) the relative effects of soaking and sprinkling; (3) the result of a possible lack of aeration and accumulation of carbon dioxid in the center of the presoak-treated mass of seeds.

A half bushel each of Dietz and China wheats in bushel bags were soaked in water 10 minutes, drained, and covered 6 hours, then soaked in formalin 1 to 320 for 10 minutes, drained, and covered 6 hours. A similar treatment was made on like quantities of seed, using copper sulphate 1 to 80 for ½ hour after the 6 hours' presoaking, followed by milk of lime. The upper and central parts of each half bushel were dried, and each of the eight lots thus obtained was planted in 10 rows of 300 seeds each. Suitable controls and seeds treated without presoaking were also planted.

At the same time, a bushel of Currell wheat, piled up on canvas, was sprinkled with water and covered for 6 hours. Half of this was then sprinkled with formalin 1 to 320 and covered for 6 hours. The other half was sprinkled with copper sulphate 1 to 80, covered ½ hour, and limed. Seeds from top and center were dried and planted, along with controls and seeds sprinkled without presoaking. The results obtained a month after planting are recorded in figure 6.

The presoak-treated seeds again showed a marked improvement in germination over seeds treated without presoaking. Seeds from the center of the bag are apparently affected to some extent, probably through lack of aeration and the accumulation of carbon dioxid; but the average germination of the presoak-treated seeds is better than that of the seeds treated without presoaking.

The sprinkling method at first sight appears to possess a distinct advantage over the soaking method. In the former, compared to controls, there is a marked increase in germination of seeds sprinkled first with water and after six hours with the disinfectant. This is most probably due to the incompleteness of the sprinkling method, since the disinfectant can not reach each kernel as in the soaking method. Hence a large number of seeds, affected only by the water vapor of the preliminary sprinkling, receive the stimulation due merely to the absorption of water and drying before planting.

EFFECT OF PRESOAK METHOD ON BACTERIUM TRANSLUCENS VAR. UNDULOSUM

EFFECT OF SIX HOURS' PRESOAKING FOLLOWED BY FORMALIN 1 TO 400 TREATMENT ON BLACKCHAFF BACTERIA ON SEEDS PLANTED ON NUTRIENT AGAR

A series of experiments was carried out parallel with the germination experiments, using 2,360 seeds, to determine whether the 6-hour formalin treatment when preceded by a 6-hour presoak would destroy or prevent the growth of the blackchaff bacteria. Heat-sterilized wheat seeds were heavily inoculated, using four virulent isolations of the blackchaff organism, and dried overnight as before described. The next day the seeds were placed in tubes of sterile tap water, which was drained off after 10 minutes. The tubes containing the seeds were placed in moist

chambers for 6 hours to maintain a thin film of moisture around each kernel throughout this period. The subsequent treament with a formalin solution, 1 to 400, in sterile tap water was made in the same manner—that is, by pouring the formalin solution on the seeds, draining if off after 10 minutes and placing the tubes in moist chambers rinsed with a formalin 1 to 400 solution. After 6 hours' treatment the seeds were replaced in sterile envelopes, dried overnight, and planted on agar plates. Control seeds, inoculated but not treated, were also planted. Observations were made after 9 to 15 days and are recorded in Table VIII.

TABLE VIII.—*Effect of 6 hours' presoaking followed by formalin 1:400 treatment on blackchaff bacteria on seeds placed on nutrient agar*

Experiment No.	Treatment.	Number of seeds used.	Percentage developing typical black-chaff colonies.	Percentage sterile.	Percentage contaminated with fungi or bacteria other than black-chaff.
I.	Inoculated seeds presoaked 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	400	0.0	65.5	34.5
	Controls inoculated but not treated	160	72.0	19.3	8.7
II.	Inoculated seeds presoaked 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	400	.0	87.3	12.7
	Controls inoculated but not treated	200	77.0	2.0	21.0
III.	Inoculated seeds presoaked 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	800	.2	97.7	2.1
	Controls inoculated but not treated	400	98.7	1.3	.0
Summary.	Inoculated seeds presoaked 6 hours, formalin 1:400 for 10 minutes, covered 6 hours	1,600	.1	87.0	12.9
	Controls inoculated but not treated	760	86.6	5.9	7.5

Only 2 out of 1,600 inoculated seeds treated by the presoak method developed typical blackchaff colonies. The controls, which had been inoculated and dried two days, showed 86.6 per cent of the kernels developing the typical colonies when planted on the nutrient agar, thus demonstrating that the absence of growth in the treated seeds was due not to drying of the bacteria but to the treatment as practiced (Pl. 79, 80). The presoaking, then, while limiting to a striking degree retardation of seed growth and loss due to failure to germinate, does not reduce the effectiveness of the subsequent formalin treatment as a means of treating diseased seed. In fact, it tends to increase its efficiency in this respect, as will be brought out in the discussion.

EFFECT OF SIX HOURS' PRESOAKING FOLLOWED BY FORMALIN 1 TO 320 AND COPPER SULPHATE 1 TO 80 ON ARTIFICIALLY INFECTED WHEAT SEEDS PLANTED IN THE SOIL UNDER FIELD CONDITIONS

Several field tests were made with seeds of Currell, China, and Dietz varieties artificially infected with blackchaff bacteria, dried, and treated with formalin 1 to 320 and copper sulphate 1 to 80, after a presoaking of six hours. The percentages of infection in the young seedlings two to three weeks after planting are given in Table IX.

TABLE IX.—*Effect of presoak method of treatment on inoculated wheat seeds under field conditions*

Treatment.	Experiment of Aug. 9.			Experiment of Aug. 22.			Experiment of Sept. 7.		
	Variety of wheat used.	Isolation number of *Bact. translucens* var. *undulosum*.	Percentage of infection of seedlings.	Variety of wheat used.	Isolation number of *Bact. translucens* var. *undulosum*.	Percentage of infection of seedlings.	Variety of wheat used.	Isolation number of *Bact. translucens* var. *undulosum*.	Percentage of infection of seedlings.
Seeds soaked in bacterial suspension 20 minutes, dried, planted at same time as treated seeds below.	Currell.	[a] 850	92.6	Currell.	[a] 271-A	85.5	Currell.	[b] 213	68.9
	China	[c] 318	90.1	China	394	91.0	China	271-A	82.0
	Dietz	[c] 394	95.1	Dietz	850	80.3	...	...	...
Seeds soaked in bacterial suspension 20 minutes, dried overnight, soaked in water 10 minutes, covered 6 hours, soaked in formalin 1:320 for 10 minutes, covered 6 hours, dried, and planted.	Currell.	850	1.0	Currell.	271-A	.7	Currell.	213	.0
	China	318	.5	China	394	.4	China	271-A	.5
	Dietz	394	.3	Dietz	850	.0	...	...	...
Seeds soaked in bacterial suspension 20 minutes, dried overnight, soaked in water 10 minutes, covered 10 hours, soaked in copper sulphate 1:80 for ½ hour, then in milk of lime 1:80 a moment, dried, and planted.	Currell.	850	.8	Currell.	271-A	.9	Currell.	213	.8
	China	318	1.5	China	394	.3	China	271-A	.6
	Dietz	394	.9	Dietz	850	.0	...	...	...

[a] From Kansas. [b] From Colorado. [c] From Montana.

Bacterial infection was prevented to a very marked degree in the treated seeds. The controls, infected with five isolations of *Bacterium translucens* var. *undulosum* from different localities, showed from 69 to 95 per cent infection, considerably more than would usually occur in naturally diseased seed, owing to the heavy artificial inoculation and brief drying period before planting. Infection of these seeds, heavily inoculated as they were, was reduced from 0 to 1.5 per cent by the presoak method, used with both formalin and copper sulphate. The application of this method, then, to the control of blackchaff on the farm is evident. The only doubt that can be entertained is in cases where the bacteria have penetrated the seed coats. Fortunately, in most such cases at least, the seeds are more or less shriveled and of light weight so that they may be screened out in advance of treatment.

RESULTS OF PRESOAK TREATMENTS ON NATURALLY INFECTED WINTER WHEAT PLANTED IN THE WHEAT FIELDS OF IOWA AND KANSAS

The first extensive field trial of this method was made in 1919 at three places in the middle western wheat belt—Ames, Iowa, and Hays and Abilene, Kans.,[1] where Kharkoff and Kanred from infected fields, screened and unscreened, was treated and drilled in after two to nine days' drying. The treatments used were (1) presoak copper-sulphate treatment, in which seeds were soaked 10 minutes in water, covered 6 hours, soaked ½ hour in copper sulphate 1 to 80, limed, dried, and planted; (2) presoak formalin treatment, in which the seeds were soaked 10 minutes in water, covered 6 hours, soaked 10 minutes in formalin 1 to 320, covered 6 hours, dried, and planted. Notes on the amount of infection on the seedlings were first made four to seven weeks after planting, since infection at this time would represent mostly primary infections due to diseased seed, before general dissemination from infection centers could set in. The results are summarized in Table X.

TABLE X.—*Preliminary results of presoak treatments of infected winter wheat in the Middle West, 1919*

Locality.	Wheat variety.	Treatment.	Size of plot.	Date treated, 1919.	Date planted, 1919.	Date observed, 1919.	Number of plants examined.	Number of plants infected.	Percentage of infection.
Ames, Iowa	Kanred [a]	No treatment, unscreened.	1/20 acre.	....	Sept. 27	Nov. 7	312	28	8.9
	..do.....	Presoak copper sulphate treatment, unscreened	...do....	Sept. 18	...do...	...do...	360	2	.6
	..do.....	Presoak formalin treatment, unscreened	...do....	...do..	...do...	...do...	343	0	.0
Hays, Kans.	Kharkoff.	No treatment, screened.	1/10 acre [b]	....	Sept. 24	Nov. 10	218	17	7.8
	Kanred..	.....do.........	...do [b] ...	....	...do...	...do...	250	23	9.2
	Kharkoff	Presoak formalin treatment screened.	...do....	Sept. 22	...do...	...do...	312	0	.0
	Kanred..	..do............	...do....	...do..	...do...	...do...	321	1	.3
Abilene, Kans	..do.....	No treatment, screened.	45 acres..	....	Oct. 6 to 10	Nov. 9	200	23	11.5
	..do.....	Presoak formalin treatment screened.	15 acres..	Oct. 1	...do...	...do...	360	[c] 0	.0

[a] A very susceptible variety.
[b] Part of main field.
[c] One diseased plant was found; but, judged by its advanced stage of growth, it was a volunteer and was therefore not from the treated seeds.

The seedlings at the time of observation bore two to five leaves, with infection visible on the first leaf of diseased plants verified by microscopic examination. In untreated areas, from 7.8 to 11.5 per cent infection was present; in treated areas, from 0 to 0.6 per cent, as shown in

[1] The author is indebted to Dr. I. E. Melhus, at Ames, and to Mr. Swanson, at Hays, for cooperation and assistance at these localities.

the table, indicating so far a satisfactory degree of control under actual field conditions through the use of the presoak method as formulated.

Plants collected from the western experimental plots March 27 to April 3, 1920, were examined microscopically for the presence of oozing bacteria in suspected blackchaff lesions and in dead leaves. Platings were made later from similar lesions where abundant oozing bacteria were found, and these developed the typical blackchaff colonies. Table XI summarizes the results obtained.

TABLE XI.—*Condition of western experimental plots in the spring of 1920*

Locality.	Treatment.	Date observed, 1920.	Number of plants examined.	Number with bacteria oozing from cut sections.	Percentage of infection.
Abilene, Kans.	Kanred wheat, untreated, 45 acres.	Mar. 27	212	46	21.7
	Presoak formalin treated plot,[a] 15 acres; plants collected from half of plot farthest from untreated area.	...do....	185	9	4.8
	Same treated plot; plants collected from other half, near untreated area.	...do....	196	12	6.1
Hays, Kans.	Kharkoff wheat, untreated	Mar. 30	141	24	17.0
	Kharkoff wheat, presoak formalin treated plot.[a]	...do....	231	7	3.0
	Kanred wheat, untreated	...do....	192	49	25.5
	Kanred wheat, presoak formalin treated plot.[a]	...do....	218	9	4.1
Ames, Iowa	Kanred wheat, untreated	Apr. 3	285	53	18.5
	Kanred wheat, presoak formalin treated plot.[a]	...do....	221	6	2.7
	Kanred wheat, presoak coppersulphate treated plot.[b]	...do....	236	5	2.1

[a] Seeds presoaked 6 hours, then treated with formalin 1 to 320 for 10 minutes, drained, covered 6 hours and dried.
[b] Seeds presoaked 6 hours, then treated with copper sulphate 1 to 80 for half hour (soaked), and dried after dipping a moment in milk of lime.

A marked increase in blackchaff was observed in the untreated plots, evidently due to wind and rain spreading the disease during the resumption of growth. The spreading effect was especially noted in the Abilene plots where the treated area adjoins the untreated. Other treated areas at Hays and Ames, more isolated, show from 2 to 4 per cent of infection, as compared with 17 to 25.5 per cent in untreated plots.

Observations made at Hays and Abilene, Kans., in the latter part of May, 1920, are summarized in Table XII. At Abilene the plants were 1 to 2 feet tall, at Hays over 2 feet high. Heads had not yet emerged. Microscopic examination and confirmation of diagnoses by platings were

made as previously indicated. Typical yellow colonies of the blackchaff organism, concentrically striated by oblique light, were readily obtained in poured plates from blackchaff leaf lesions, which at this stage appeared characteristically as brown, water-soaked linear areas, narrow and extending for various lengths along the edges or centers of the second, third, or fourth leaves from the top, with clouds of oozing bacteria in cut sections. Septoria was also found in the oldest leaves, distinguished by wider lesions and characteristic black dots of pycnidia.

TABLE XII.—*Condition of experimental plots in May, 1920*

Locality and date.	Plot.	Number of plants examined.	Number with bacteria oozing from cut sections.	Percentage of infection.
Abilene, Kans., May 15, 1920.	Kanred, untreated	312	87	27.8
	Kanred, western area, presoak formalin treated.	386	26	6.7
Hays, Kans., May 22, 1920.	Kanred, untreated	293	98	33.4
	Kanred, presoak formalin treated	356	33	9.2
	Kharkoff, untreated	266	72	27.0
	Kharkoff, presoak formalin treated	298	22	7.3

There is an evident increase in the amount of secondary infection during April and May. It was also observed that most of the lesions on leaves from treated plots were small, 2 to 8 mm. long, consisting of from 1 to 3 spots on the second or third leaf from the top, and were evidently fairly recent infections. Leaves from control plots showed similar lesions but also a larger proportion of more advanced lesions up to 30 mm. long on the older leaves. No lesions were observed in the young heads, which were still inclosed in the sheath.

EFFECT OF MODIFYING THE TREATMENT PERIODS UNDER FIELD CONDITIONS

A shortening of the entire treatment period appeared desirable after field experience with the method so far described, mainly for the purpose of facilitating drying after treatment. Three greenhouse germination experiments were made with Currell wheat seed, using a shorter presoak time and a longer (varying) soak in formalin 1 to 320, followed by very short periods during which the seeds were kept moist, the entire process covering various periods from 5½ to 8 hours as outlined in figure 7.

Formalin treatments involving a soaking longer than previously used—that is, of 15 to 30 minutes—followed by immediate drying or involving a short moist period of 1 to 3 hours decreased the germination considerably. The same treatments preceded by a 5-hour presoak (in one case a 6-hour presoak) resulted in no injury whatever to germination and in fact caused distinct acceleration. The effect of the various periods upon germination was determined for infected wheat seed also.

Currell wheat seed was inoculated with a bacterial suspension of *Bacterium translucens* var. *undulosum*, isolation No. 850, treated with formalin for the periods given in Table XIII, planted outdoors, and the percentage of infected seedlings determined 27 days after planting.

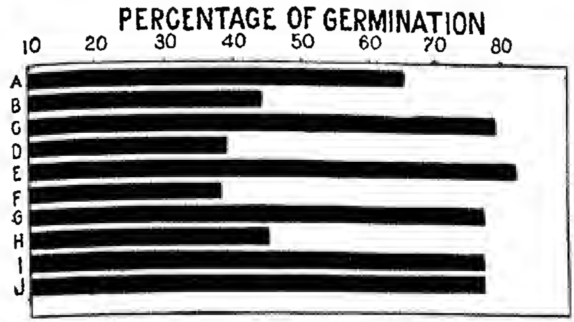

FIG. 7.—Graph showing effect of formalin 1 to 320 treatments for various periods, with and without presoaking: A, control, untreated; B, seeds soaked in formalin 1 to 320 for 15 minutes drained, covered 2 hours, dried overnight, and planted; C, seeds soaked in water 10 minutes, covered 5 hours, then treated with formalin as in B; D, seeds soaked in formalin 1 to 320 for 15 minutes, drained, covered 3 hours, and dried overnight; E, seeds soaked in water 10 minutes, covered 5 hours, then treated with formalin as in D; F, seeds soaked in formalin 1 to 320 for 30 minutes, drained, covered 1 hour, and dried overnight; G, seeds soaked in water 10 minutes, covered 5 hours, then treated with formalin as in F; H, seeds soaked in formalin 1 to 320 for 30 minutes, drained, and dried overnight; I, seeds soaked in water 10 minutes, covered 5 hours, then treated with formalin as in H; J, seeds soaked in water 10 minutes, covered 6 hours, then treated with formalin as in H. Records of germination were made on the sixth day after planting and are the averages of three experiments.

TABLE XIII.—*Effect of shortened presoak method of treatment on infected wheat seed under field conditions*

Treatment.	Number of plants examined.	Number of plants infected.	Percentage of infection after 27 days.
Inoculated seeds dried and planted without further treatment	221	40	18.2
Inoculated seeds soaked in water 10 minutes, drained, covered 5 hours, soaked in formalin 1:320 for 15 minutes, covered 2 hours, dried, and planted	215	4	1.8
Inoculated seeds soaked in water 10 minutes, drained, covered 5 hours, soaked in formalin 1:320 for 15 minutes, covered 3 hours, dried, and planted	196	1	.5
Inoculated seeds soaked in water 10 minutes, drained, covered 5 hours, soaked in formalin 1:320 for 30 minutes, covered 1 hour, dried, and planted	231	0	0
Inoculated seeds soaked in water 10 minutes, drained, covered 5 hours, soaked in formalin 1:320 for 30 minutes, dried, and planted	211	2	.9
Inoculated seeds soaked in water 10 minutes, drained, covered 6 hours, soaked in formalin 1:320 for 30 minutes, dried, and planted	246	3	1.2

The best control in this experiment was obtained with a presoak of 5 hours, followed by 30 minutes' formalin soak, then covering 1 hour. Such a process, requiring 6½ hours in all, would be particularly desirable because of the ease in subsequently drying the seeds by spreading them in the sun on the day of treatment. Further field repetition of this experiment, which was suspended at this time by the advent of winter,

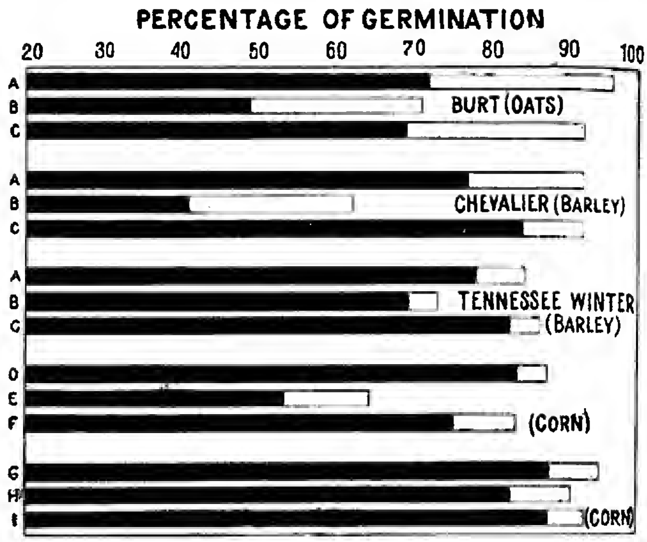

FIG. 8.—Graph showing effect of formalin 1 to 320 and 1 to 200 on germination of corn, barley, and oats with and without presoaking: A, controls; B, seeds soaked in formalin 1 to 320 for 10 minutes, drained, kept moist (covered) 6 hours, dried overnight, and planted; C, seeds soaked in water 10 minutes, drained, and kept moist (covered) 6 hours, then soaked in formalin 1 to 320 for 10 minutes, drained, and kept moist (covered) 6 hours, dried overnight, and planted; D and G, controls; E and H, seeds soaked in formalin 1 to 200 for 10 minutes, drained, kept moist (covered) 4 hours, dried overnight, and planted; F, seeds soaked in water 10 minutes, drained, kept moist (covered) 10 hours, then soaked in formalin 1 to 200 for 10 minutes, drained, kept moist (covered) 4 hours, dried overnight, and planted; I, seeds soaked in water 10 hours, drained thoroughly a few minutes, then soaked in formalin 1 to 200 for 10 minutes, drained, kept moist (covered) 4 hours, dried overnight, and planted. Records of the germination were made on the fifth and seventh days after planting in the greenhouse.

is necessary, however, before definite recommendations on this modification can be made.

EFFECT OF THE PRESOAK METHOD ON OTHER CEREALS

The uniform results obtained on nine different varieties of wheat by the presoak method of treatment with formalin and copper sulphate and a consideration of the underlying principles governing its salutary action, as will be discussed later, suggested that it might be generalized for the treatment of all seed-transmitted diseases of economic importance amen-

able to control by formalin and copper sulphate. So far, this method has been tested on the germination of oats, barley, and maize with results similar to those obtained for wheat. Copper-sulphate injury can be prevented for Tennessee winter barley as shown above. The results thus far obtained with the presoak method of treatment, using formalin 1 to 320 on oats and barley and formalin 1 to 200 on maize, are given in figure 8. Oats and barley were soaked in water 10 minutes, drained, and kept moist 6 hours, then soaked in formalin 1 to 320 for 10 minutes, and covered 6 hours. Maize, which absorbs water much more slowly than wheat, oats, or barley and is also less susceptible to formalin injury, was given a 10-hour presoak—that is, 10 minutes in water, draining and covering for 10 hours, followed by 4 hours' formalin 1 to 200 treatment.

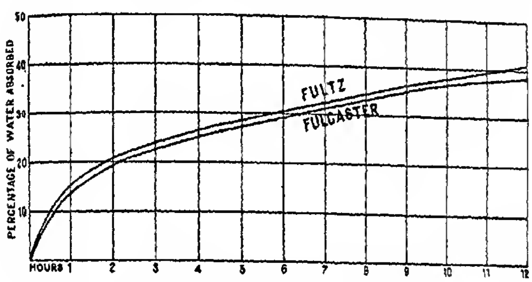

FIG. 9.—Curve showing rate of absorption of water by dry wheat seeds.

In another case it was given a 10-hour actual soaking in water, followed by the treatment.

The presoak method is evidently applicable to these cereals also, as a means of preventing seed injury due to disinfectants (Pl. 81, 82); and the possibility of its application for other kinds of seeds is obvious.

GENERAL DISCUSSION

As a result of these experiments several facts stand out clearly. First, in all cases, with each variety of wheat, barley, oats, and maize tested, the presoak method minimized or eliminated the injury to seed germination due to the use of formalin and copper sulphate. Second, as shown in the illustrations, a marked stimulation of growth was usually produced. Third, the presoak method proved fully efficient as a means of destroying or preventing the growth of the bacteria of the blackchaff disease borne on the seed and can undoubtedly be applied for the prevention of other diseases. Fourth, the method is simple and adapted to field conditions, since any farmer can apply it.

The cause of the first-mentioned effect of the presoak treatment may be partly accounted for upon an analysis of figure 9. This represents the rate of absorption of water by 10 gm. each of dry seeds of two wheat

varieties, soaked 10 minutes in water, drained, and kept moist for 12 hours, with periodical weighings after blotting off all surface water each time. The excess weight above 10 gm. represents the amount of water absorbed. The curve rises rapidly in the first 3 hours, then slows down somewhat to a more gradual rise. At the end of 6 hours, about 30 per cent by weight of water has been absorbed; during the next 6 hours, about 10 per cent more is absorbed. The 6 hours' presoaking, as practiced, consequently impregnates the cell walls and cells of the seed with water, increasing the size and adding about 30 per cent by weight to the seeds. The next 6 hours' treatment with water containing formalin in solution adds only one-third as much more; and the formalin solution as it diffuses into the seeds is consequently greatly diluted by the amount of water already present in the tissues. Moreover, the amount of formalin solution which can enter the tissues in the 6 hours after presoaking is only one-third of what enters during a 6-hour formalin treatment without presoaking. Should the subsequent formalin treatment last much longer than 6 hours, an equilibrium would finally be established between the strength of solution within the seeds and that on the surface, resulting in both cases in a solution weaker than the original, in accordance with the laws of diffusion of dissolved substances. Removing the presoaked seeds after 6 hours' formalin treatment leaves them with a solution content considerably more dilute than that finally present in air-dry seeds directly treated with the full-strength solution; consequently the weakened solution within the presoaked seeds resulted in a very marked decrease in seed injury, as observed throughout the experiments.

As for the stimulation observed in presoaked seeds, this may be due partly or wholly to the well-known stimulating effect of a toxic agent in minimum dose, such as would finally be present in the presoaked seeds.

In considering the effect of the presoak method on the blackchaff bacteria on the seed coat, the dominant factor involved is the established principle that microorganisms in an active vegetative condition are more susceptible to the action of destructive agents than when dormant. Presoaking the seeds, and consequently the bacteria on them, for a period of six hours at room temperature causes the bacteria to begin to resume vegetative activity before seed germination commences, because the moisture and temperature conditions are ample for bacterial growth and division to begin during this period. Subsequently exposed to the direct action of the formalin solution applied full strength to the surface of the seeds, the bacteria are naturally much more susceptible to destruction in this active condition. As a result, the disinfectant must act with greater efficiency than in the usual treatment, where it acts on dried and dormant bacteria. The six hours' presoak, on the other hand, is not sufficient to cause wheat-seed germination, which would produce a condition extremely susceptible to formalin injury.

The method of treatment discussed has, therefore, a two-fold advantage. On the one hand, wheat-seed injury due to the use of formalin and copper sulphate is eliminated or reduced to a minimum. On the other hand, the blackchaff organisms on the seed coats are rendered particularly sensitive to the action of the disinfectant by being previously brought into a vegetative condition.

The same physiological principles discussed above should hold true for the general problem of seed treatment for various seed-borne pathogens. The consistency of the results obtained by this method with nine varieties of wheat and with other cereals, using formalin and copper sulphate, indicates the possibility of the use of the presoak method with other kinds of seeds as a means of minimizing or preventing seed disinfectant injury. Similarly, other pathogenic organisms, bacteria, or fungus spores, may be stimulated by the presoak method into increased susceptibility to the disinfectant.

The presoak method of seed treatment with chemical disinfectants may be formulated for general purposes as consisting of two parts: First, the presoak period, in which seeds are soaked in water for 10 minutes, drained, and kept covered and moist for a definite period of time, which is 6 hours for wheat, barley, and oats and 10 to 18 hours for maize—in no case sufficient to begin seed germination; second, the disinfectant-treatment period immediately following, in which the disinfectant is applied exactly as now practiced. The relative time of the presoak and subsequent treatment for other diseases, probably varying with each kind of seed and pathogen, is dependent on the following factors:

(1) Susceptibility of the kind of seed used to the disinfectant.

(2) Susceptibility of the pathogen to the disinfectant.

(3) Rate of absorption of water by the seeds.

(4) Time at which seed germination begins.

(5) Time at which vegetative activity of the pathogen begins.

A proper balance of these factors must be obtained, such that the optimum seed germination and the optimum germicidal efficiency are secured, as reported for the blackchaff disease of wheat.

The length of the presoak period should not exceed half or two-thirds of the period necessary for seed germination to begin, since germination before treatment with the disinfectant would result in extreme sensitiveness to injury. On the other hand, the pathogen, especially if bacterial in nature, usually has a much shorter germination period, which should come within the limit of the time of presoak and thus render it susceptible long before the seed has begun to germinate. The period necessary for the absorption of about 30 per cent by weight of water appears to be sufficient, and in the case of the cereals so far tried seems to counteract disinfectant injury. In wheat, oats, and barley this is five to six hours. The length of time necessary for other kinds of seeds to absorb about 30 per cent of water is suggested as the presoak period when not conflicting with the other factors involved.

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Actual soaking in water throughout the presoak period does not appear to be so favorable for wheat-seed treatment as the procedure of soaking 10 minutes in water and merely keeping moist for 6 hours.

For use in farm practice this method does not involve any radical change in present procedure other than to keep the seeds moist for a definite time before treating. In controlling the blackchaff disease of wheat, seeds should first be screened to remove shriveled grain. Then the seeds in sacks or bags, in quantities of not more than ½ bushel each, can be soaked early in the morning in water for 10 minutes, drained, and set away in the bags while moist. Six hours later, at about noon, the seeds should be thoroughly soaked for 10 minutes in a formalin solution of 1 pound to 40 or 50 gallons of water, drained, and left in the bags for 6 hours. In the evening the seeds should be spread out to dry overnight and are ready for planting the next morning.

The use of formaldehyde vapor recently proposed by Thomas (*10*) for seed treatment, while eminently suitable for the disinfection of small seed lots which are not to be planted immediately, is open to the serious objection of lack of penetration throughout the seed mass and is not so well adapted as the presoak method for the treatment of seeds in large masses in farm practice. His experiments indicate that the vapor, while efficient on surface seeds, does not reach seeds at a depth of ½ inch, so that these remain as badly contaminated as untreated controls. In the presoak method, every seed is surrounded by a film of the disinfectant acting on the pathogens which previously have been brought into a vegetative condition by the long exposure to moisture at room temperature.

The presoak method used with copper sulphate, if efficient for controlling the cereal smuts,[1] would be particularly adapted for the grain sections of the Northwest. Extensive soil infection in this area renders the use of copper sulphate preferable to formalin because of its residual germicidal effect; and, as here shown, copper-sulphate injury may be prevented by a 6-hour presoak.

The general application of the presoak method, extremely simple in itself, to the formalin and copper-sulphate treatments of the cereal diseases amenable to control by seed disinfection should, if the results here recorded are confirmed for other diseases by subsequent careful

[1] A paper by Heald (*2*), first brought to the writer's attention in Nov. 14, 1919, when these experiments were completed and the manuscript was prepared for the press, shows some interesting data on a somewhat similar method used for treatment of barley smut. Heald soaked barley seeds in water 4 hours, covered them 8 hours longer, then treated them with formalin 1 to 288 for 10 minutes and then kept them covered 2 hours. No statement as to the manner of arriving at the use of this procedure is made. His figures indicate for this treatment (1) less injury to germination than for any other formalin treatment which he used, (2) effective control of barley smut—0.93 per cent smut in a plot treated in this manner and 0.73 per cent smut in a somewhat similarly treated copper-sulphate plot compared to an average of 33.02 per cent smut in three untreated plats. This corroborates for barley smut the work reported on blackchaff with the presoak method. Heald does not appear to have followed up his work, which was clearly a rule of thumb, nor did he recommend this particular method for general use with other methods. He made no allowance for loss in number of seeds per bushel through swelling, otherwise he must have obtained results which would have indicated to him clearly the importance of the method, since he must then have obtained larger yields than by any other method which he used. Moreover, my method differs from Heald's in that it gives only a short plunge in water rather than a long one, and this is an important difference.

research, result in a saving of a large percentage of seeds destroyed by the usual treatments or delayed in germination and thus longer exposed to the attack of soil fungi, giving at the same time a more efficient germicidal action on the pathogens involved.

SUMMARY

(1) The use of formalin and copper sulphate as now practiced usually causes retardation and injury to seed germination.

(2) Greenhouse and field experiments here reported have shown that this detrimental effect can be eliminated for standard varieties of wheat by allowing the seeds to absorb water for six hours before submitting them to the treatment with formalin or copper sulphate. Soaking for a short period (10 minutes) and covering for 6 hours, here designated the presoak method, is better than leaving in water for 6 hours. Similar results were obtained in experiments with barley, oats, and corn.

(3) The saturation of the seed cells and cell walls with water during the presoak period appears to be the factor counteracting the injurious effect on seed germination by diluting the disinfectant beyond the point of injury as it diffuses into the tissues and also by considerably decreasing the amount of water plus disinfectant solution which may enter the tissues after presoaking as compared to what may enter without any presoaking.

(4) Actual stimulation of germination has been observed repeatedly in presoak-treated seeds, a factor which by shortening germination minimizes the danger of exposure to the attack of soil organisms during this susceptible period.

(5) The bacterial blackchaff disease of wheat can be controlled without any injury to seed germination by a 6-hour presoak of surface-infected seeds in water followed by a 6-hour treatment with formalin 1 to 400 in the manner prescribed.

(6) In practice, wheat seeds after being screened should be soaked with water for 10 minutes at about 6 o'clock in the morning, drained, covered, and set away moist till noon, then soaked with formalin 1 to 400 for 10 minutes, drained, covered, and set away moist till 6 o'clock in the evening, when they should be spread out to dry overnight to be ready for planting the next day.

(7) In planting, an allowance must always be made for the fact that there are fewer treated seeds in a bushel than there are of dry untreated ones. In general, it is recommended to sow about 25 per cent more bulk than is usual of the dry grain, otherwise fewer seeds will be actually planted and the yield will be reduced correspondingly.

(8) The use of the presoak method tends to increase the efficiency of the disinfectant, in that the presoaking stimulates dormant bacteria and possibly fungi into vegetative activity, thereby rendering them extremely susceptible to the subsequent action of the disinfectant.

(9) The general use of the presoak method of treatment in farm practice for other diseases involves no radical change in present procedure,

the only deviation being to keep the seeds moist for a definite period before giving them the disinfectant treatment.

(10) In applying the principles here utilized to other kinds of seeds, the determination of the lengths of the two parts of this method—(1) the presoak period, (2) the subsequent disinfectant treatment period—must be governed by the following factors: (a) the rate of absorption of water by the seeds, (b) the susceptibility of the seeds and pathogens to the disinfectant, and (c) the respective periods necessary for the beginning of seed germination and of vegetative activity of the pathogen. In no case must the presoak period be continued until seed germination begins. The length of time necessary for the seeds to absorb about 30 per cent of their weight of water is suggested as the length of the presoak period when not conflicting with the other factors involved.

(11) The presoak method of treatment, as here formulated, is proposed as a basis for the reinvestigation of practical seed treatment for all seed-transmitted diseases of economic importance amenable to control by formalin and copper sulphate as a means of eliminating seed injury and at the same time increasing germicidal efficiency.

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PLATE 69

Relative injury to wheat-seed germination caused by short and long formalin treatments:

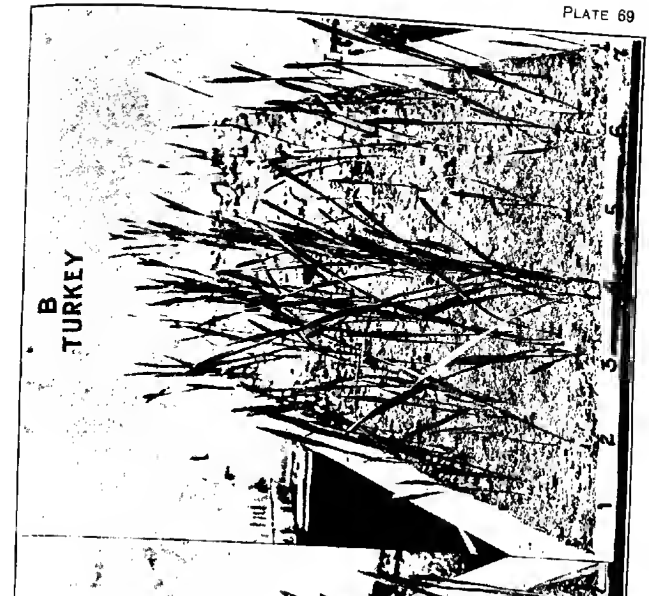
A.—Effect of formalin 1 to 400 and 1 to 200 treatment for 3, 6, and 12 hours on China variety:

Row 1, formalin 1 to 400 for 3 hours, 56 per cent germination;
Row 2, formalin 1 to 400 for 6 hours, 53 per cent germination;
Row 3, formalin 1 to 400 for 12 hours, 74 per cent germination;
Row 4, control, 78 per cent germination;
Row 5, formalin 1 to 200 for 3 hours, 39 per cent germination;
Row 6, formalin 1 to 200 for 6 hours, 37 per cent germination;
Row 7, formalin 1 to 200 for 12 hours, 57 per cent germination.

B.—Effect of formalin 1 to 400 and 1 to 200 treatment for 3, 6, and 12 hours on Turkey variety:

Row 1, formalin 1 to 400 for 3 hours, 42 per cent germination;
Row 2, formalin 1 to 400 for 6 hours, 48 per cent germination;
Row 3, formalin 1 to 400 for 12 hours, 50 per cent germination;
Row 4, control, 56 per cent germination;
Row 5, formalin 1 to 200 for 3 hours, 23 per cent germination;
Row 6, formalin 1 to 200 for 6 hours, 17 per cent germination;
Row 7, formalin 1 to 200 for 12 hours, 34 per cent germination.

Note increased vigor and germination of 12-hour treated seeds compared with 3-hour or 6-hour treated seeds.


A
CHINA
B
TURKEY

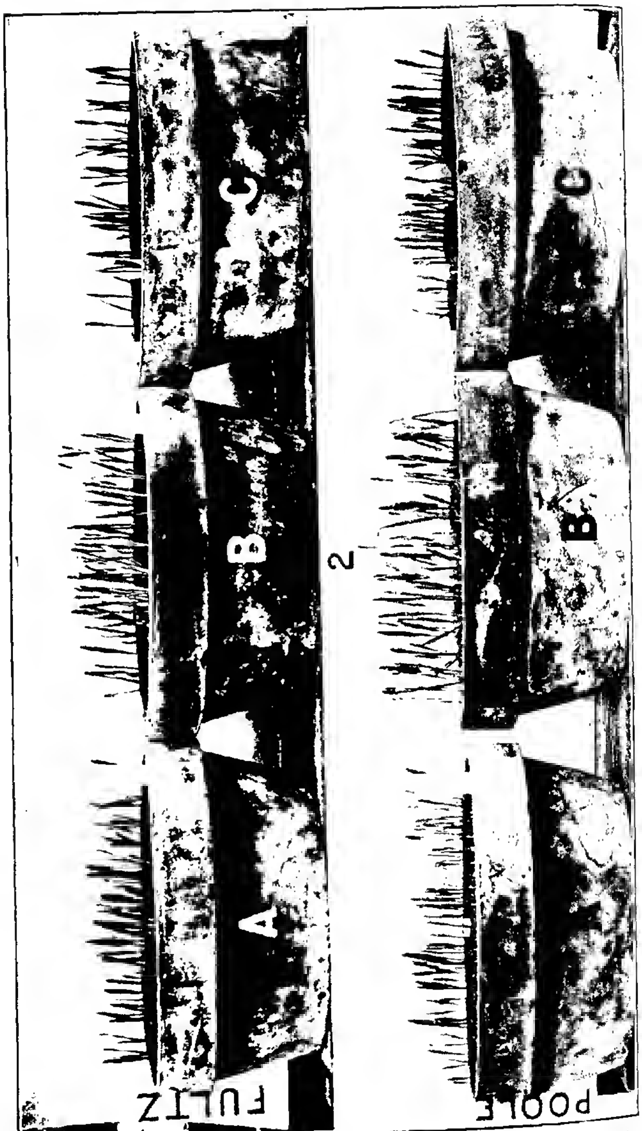

FULTZ
POOLE
A
B
C
2

PLATE 70

Effect of formalin 1 to 400 treatment for 6 hours, with and without 6-hour presoak:

1. Fultz wheat: A, control, 76 per cent germination; B, seeds presoaked 6 hours, then formalin 1 to 400 for 6 hours, 79 per cent germination; C, seeds treated with formalin 1 to 400 for 6 hours, not presoaked, 57 per cent germination, plants dwarfed.

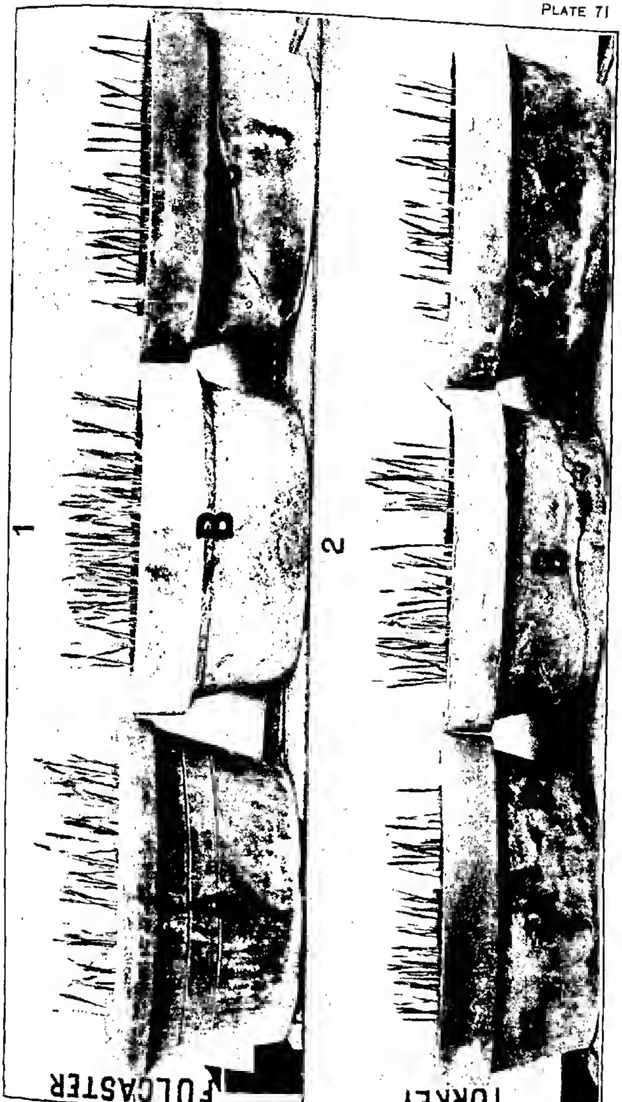
2. Poole wheat: A, control, 90 per cent germination; B, seeds presoaked 6 hours, then formalin 1 to 400 for 6 hours, 89 per cent germination; C, seeds treated with formalin 1 to 400 for 6 hours not presoaked, 67 per cent germination, plants dwarfed.

PLATE 71

Effect of formalin 1 to 400 treatment for 6 hours, with and without 6-hour presoak.

1. Fulcaster wheat: A, control, 71 per cent germination; B, seeds presoaked 6 hours, then formalin 1 to 400 for 6 hours, 83 per cent germination; C, seeds treated with formalin 1 to 400 for 6 hours, not presoaked, 54 per cent germination, plants dwarfed.

2. Turkey wheat: A, control, 61 per cent germination; B, seeds presoaked 6 hours then formalin 1 to 400 for 6 hours, 68 per cent germination; C, seeds treated with formalin 1 to 400 for 6 hours without presoaking, 50 per cent germination, plants dwarfed.


1
B
2
FULCASTER
TURKEY

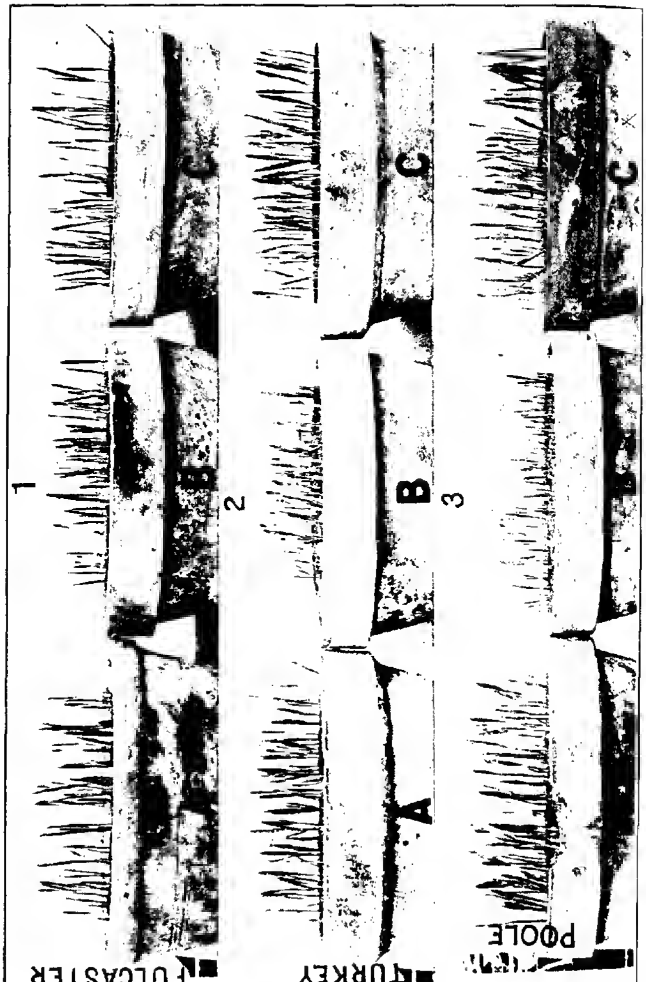

FULCASTER
TURKEY
POOLE
1
2
3
A
B
C

PLATE 72

Stimulating effect of the presoak method of treatment with formalin 1 to 400. (Repetition of experiments shown in Pl. 70, 71):

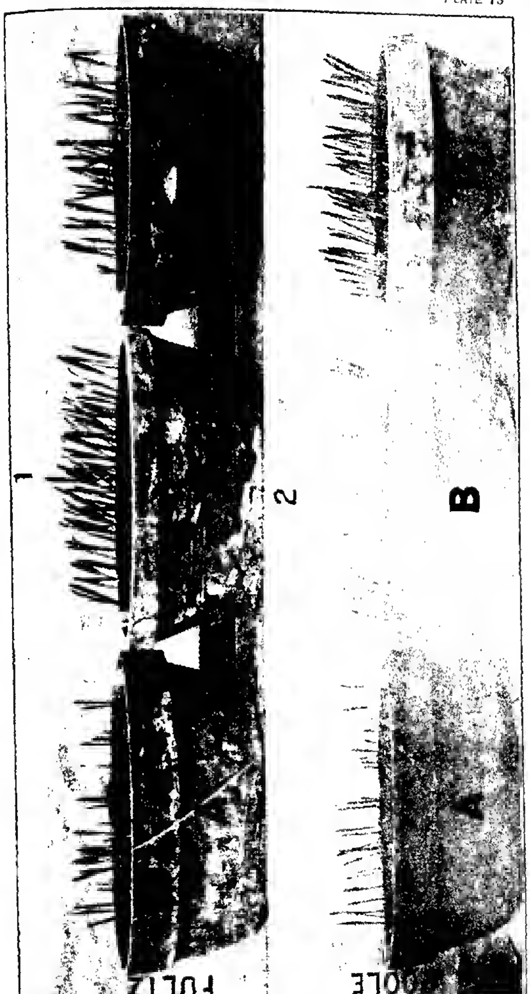
1. Fulcaster wheat: A, C, seeds presoaked 6 hours, then formalin 1 to 400 for 6 hours, 84 per cent germination, plants stimulated; B, control, 87 per cent germination.
2. Turkey wheat: A, C, seeds presoaked 6 hours, then formalin 1 to 400 for 6 hours, 64 per cent germination; B, control, 67 per cent germination. Note stimulation in presoak-treated plants.
3. Poole wheat: A, C, seeds presoaked 6 hours, then formalin 1 to 400 for 6 hours, 88 per cent germination; B, control, 94 per cent germination. Note increased vigor and stimulation in presoak-treated plants.

PLATE 73

Effect of formalin 1 to 200 treatment for 6 hours, with and without 6-hour presoak:

1. Fultz wheat: A, seeds treated with formalin 1 to 200 for 6 hours without presoak, 43 per cent germination; B, control, 86 per cent germination; C, seeds presoaked 6 hours, then formalin 1 to 200 for 6 hours, 70 per cent germination.

2. Poole wheat: A, seeds treated with formalin 1 to 200 for 6 hours without presoak, 46 per cent germination; B, control, 81 per cent germination; C, seeds presoaked 6 hours, then formalin 1 to 200 for 6 hours, 71 per cent germination. Note stimulation in presoak-treated plants.



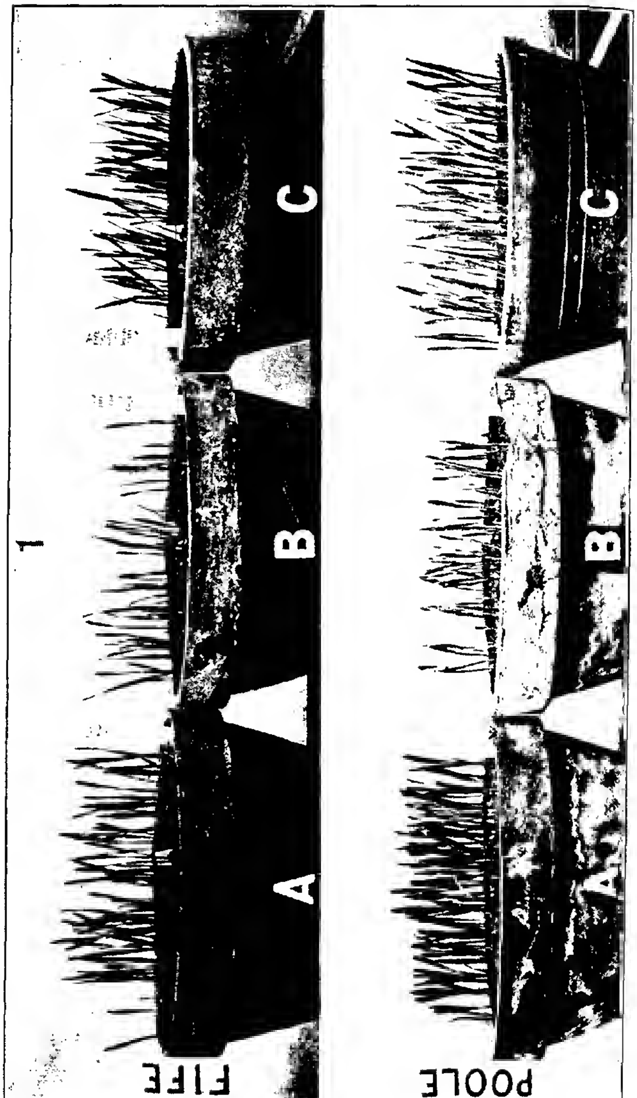

1
A
B
C
FIFE
A
B
C
POOLE

PLATE 74

Effect of formalin 1 to 320 treatment for 6 hours, with and without 6-hour presoak:

1. Fife wheat: A, control, 79 per cent germination; B, seeds treated with formalin 1 to 320 for 6 hours, 53 per cent germination; C, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 82 per cent germination.

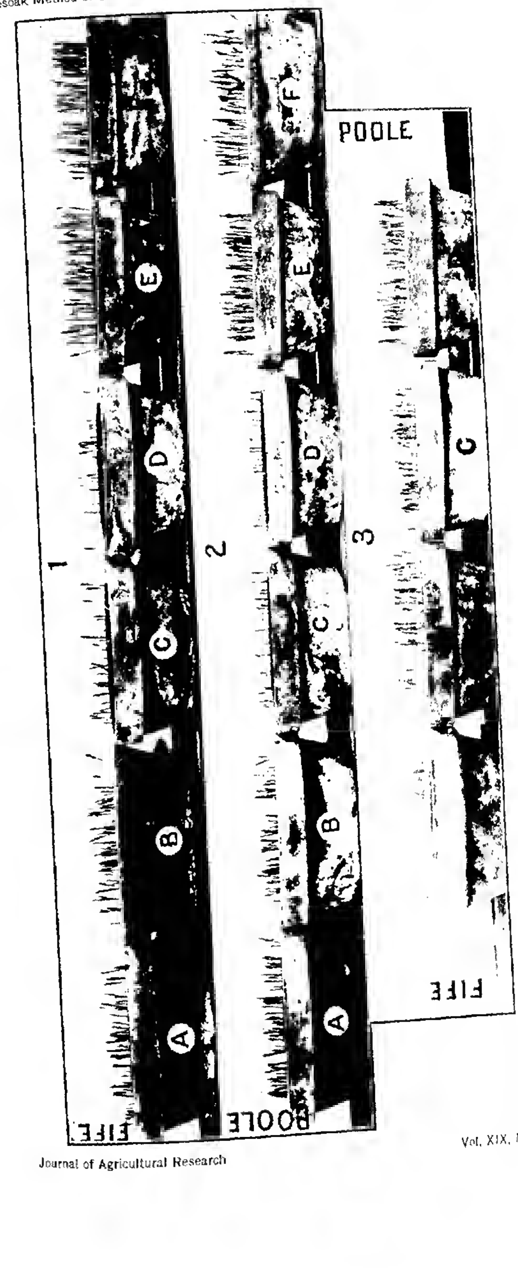
2. Poole wheat: A, control, 88 per cent germination; B, seeds treated with formalin 1 to 320 for 6 hours, 70 per cent germination; C, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 86 per cent germination.

PLATE 75

1. Fife wheat: A, B, control, 83 per cent germination; C, D, seeds treated with formalin 1 to 320 for 3 hours, 62 per cent germination; E, F, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 90 per cent germination.

2. Poole wheat: A, B, control, 85 per cent germination; C, D, seeds treated with formalin 1 to 320 for 6 hours, 55 per cent germination; E, F, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 88 per cent germination.

3. Effect of soaking in water throughout presoak period, compared with procedure of keeping moist 6 hours: A, B, Fife wheat; C, D, Poole wheat; A, C, seeds soaked in water 5 hours, then treated with formalin 1 to 320 for 7 hours; B, D, seeds soaked in water 10 minutes, drained, and kept moist 6 hours, then treated with formalin 1 to 320 for 6 hours. Note the greater stimulation in B and D.


1
2
3
FIFE
POOLE
POOLE
FIFE
A
B
C
D
E
F

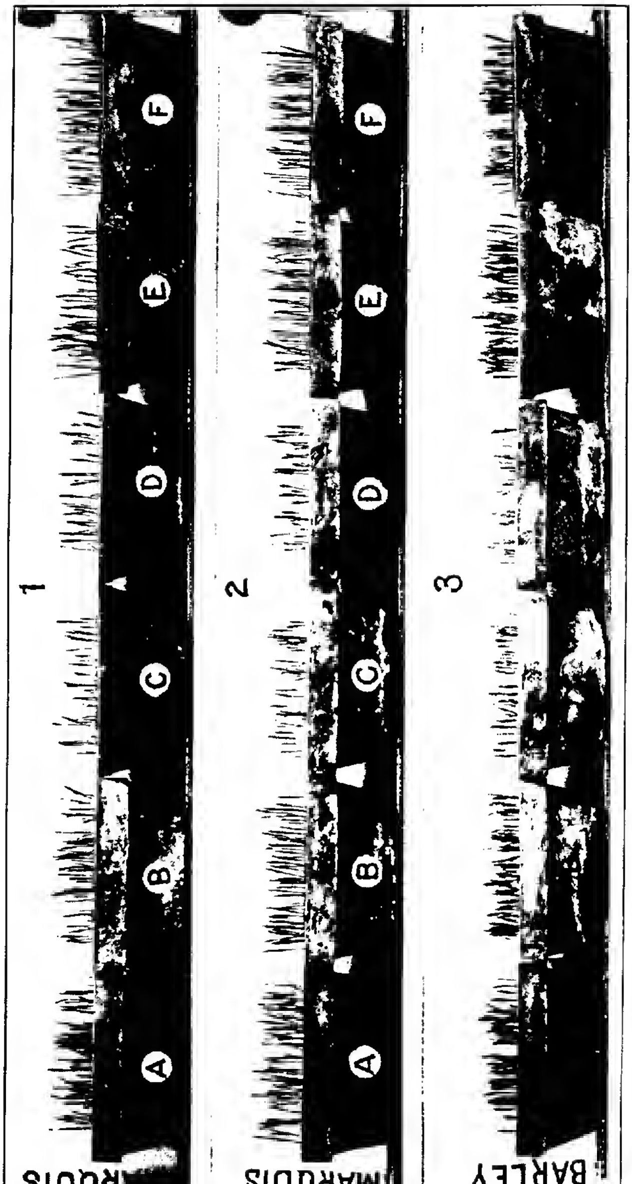

1
2
3
MARQUIS
MARQUIS
BARLEY
A
B
C
D
E
F

PLATE 76

Effect of formalin and copper sulphate on wheat and of copper sulphate on barley, with and without presoaking:

1. Marquis wheat: A, B, control, 76 per cent germination; C, D, seeds treated with formalin 1 to 320 for 6 hours, 57 per cent germination; E, F, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 77 per cent germination.

2. Marquis wheat: A, B, control, 84 per cent germination; C, D, seeds treated with copper sulphate 1 to 80 for ½ hour, and limed, 65 per cent germination; E, F, seeds presoaked 6 hours, then copper sulphate 1 to 80 for ½ hour, and limed, 79 per cent germination.

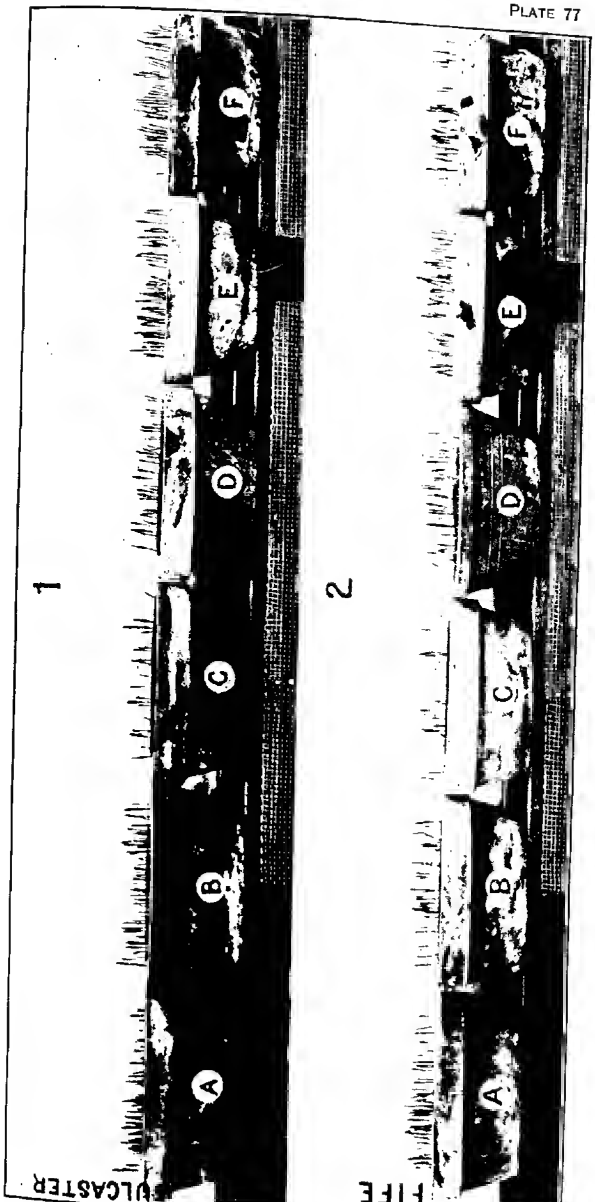
3. Tennessee winter barley: A, B, control, 89 per cent germination; C, D, seeds treated with copper sulphate 1 to 80 for ½ hour, and limed, 72 per cent germination; E, F, seeds presoaked 6 hours, then copper sulphate 1 to 80 for ½ hour, and limed, 83 per cent germination.

PLATE 77

Effect of presoak method used with copper-sulphate treatment of wheat:

1. Fulcaster wheat: A, B, control, 81 per cent germination; C, D, seeds treated with copper sulphate 1 to 80 for ½ hour, and limed, 57 per cent germination; E, F, seeds presoaked 8 hours, then copper sulphate 1 to 80 for ½ hour, and limed, 82 per cent germination.

2. Fife wheat: A, B, control, 79 per cent germination; C, D, seeds treated with copper sulphate 1 to 80 for ½ hour, and limed, 52 per cent germination; E, F, seeds presoaked 8 hours, then copper sulphate 1 to 80 for ½ hour, and limed, 81 per cent germination.


1
2
FIFE
A
B
C
D
E
F

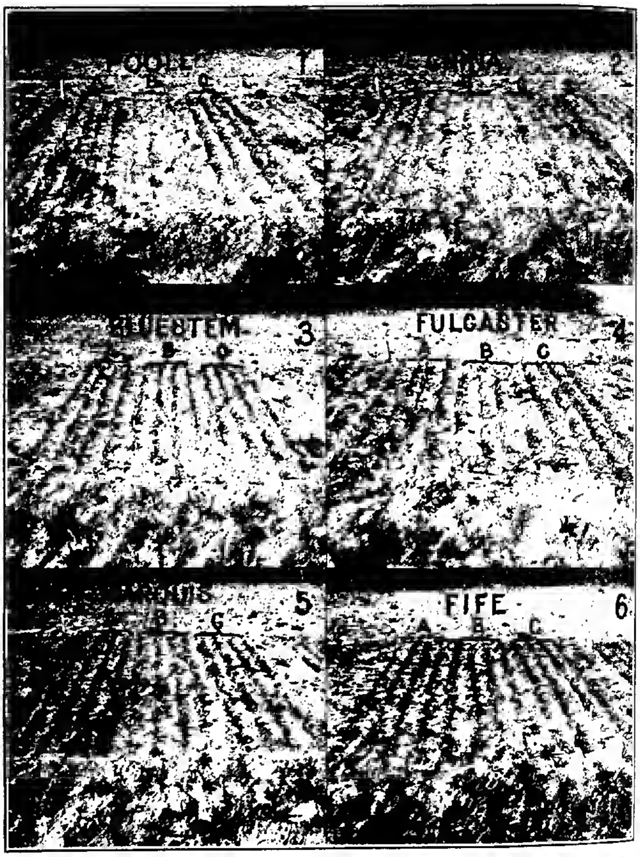

1
B
C
2
BLUESTEM
3
B
C
FULCASTER
4
B
C
5
B
C
FIFE
6
A
B
C

PLATE 78

Effect of presoak method used with formalin 1 to 320 on wheat under field conditions: 1. Poole; 2. China; 3. Bluestem; 4. Fulcaster; 5. Marquis; 6. Fife.

A.—Eight hundred seeds in four rows, soaked in water 10 minutes, kept moist 6 hours, soaked in formalin 10 minutes, kept moist 6 hours, dried overnight.

B.—Eight hundred seeds in four rows, soaked in formalin 1 to 320 for 10 minutes, kept moist 6 hours, dried overnight.

C.—Eight hundred seeds in four rows, control.

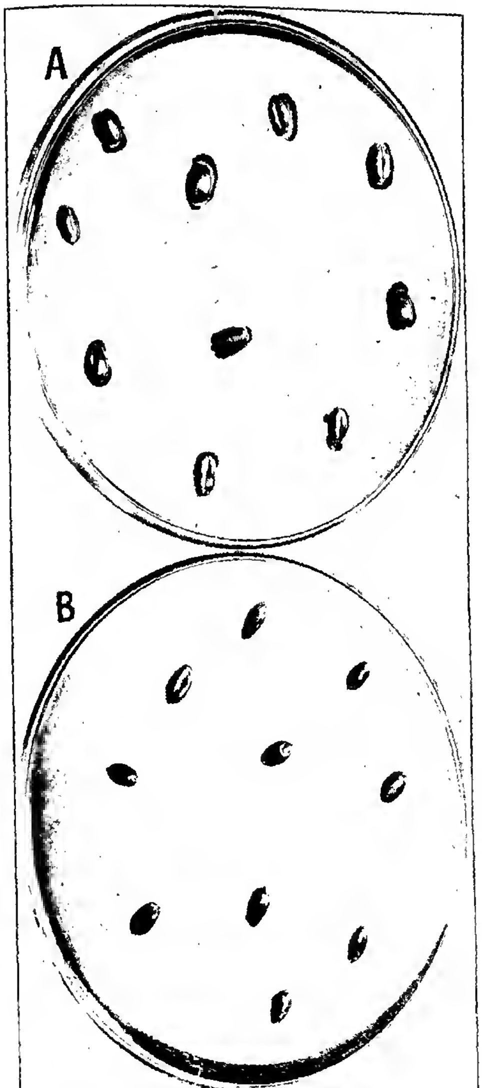
PLATE 79

Effect of presoak method used with formalin 1 to 400 on blackchaff bacteria:

A.—Controls, dry-heat sterilized wheat seeds inoculated with blackchaff isolation No. 377 from South Dakota, dried, and planted on agar without further treatment.

B.—Sterilized wheat seeds inoculated with isolation No. 377, dried overnight, then soaked in sterile tap water 10 minutes and kept moist 6 hours, then soaked in formalin 1 to 400 for 10 minutes and kept moist 6 hours, dried, and planted.

Note bacterial growth around untreated seeds and absence of growth in presoaked formalin-treated seeds. Two Petri dishes photographed out of a set of 120 dishes in experiment III on tenth day.


A
B

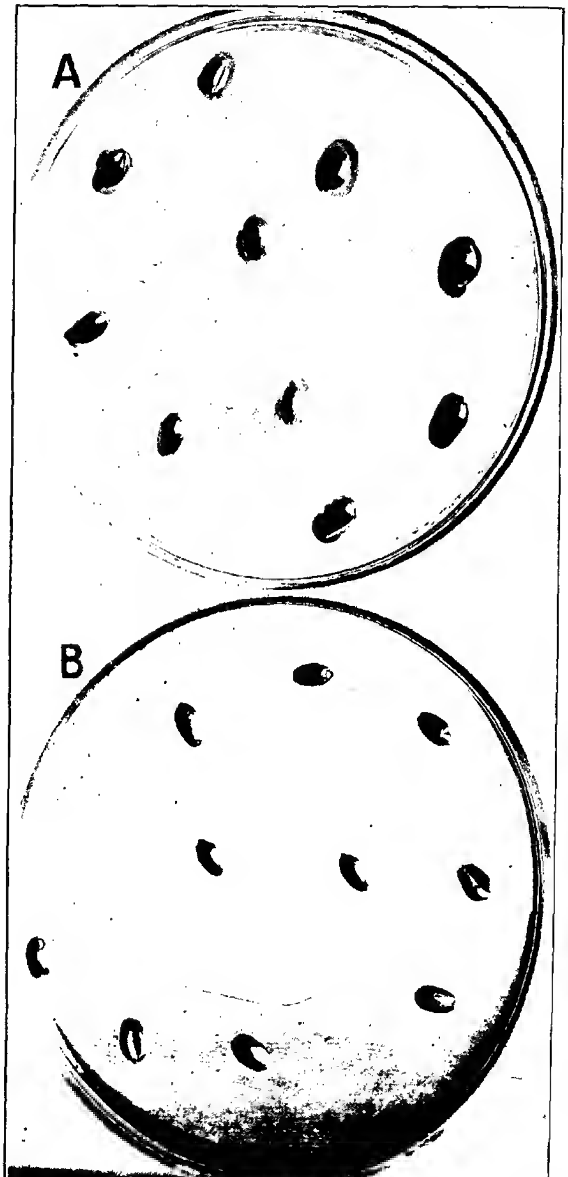


PLATE 80

Effect of presoak method used with formalin 1 to 400 on blackchaff bacteria:

A.—Controls, dry-heat sterilized wheat seeds inoculated with blackchaff isolation No. 373 from North Dakota, dried, and planted on agar without further treatment.

B.—Sterilized wheat seeds inoculated with isolation No. 373, dried overnight, then soaked in sterile tap water 10 minutes, kept moist 6 hours, then soaked in formalin 1 to 400 for 10 minutes, drained, kept moist 6 hours, dried, and planted.

Note bacterial growth around untreated seeds and absence of growth in presoaked formalin-treated seeds. Two Petri dishes photographed out of a set of 120 dishes in experiment III on tenth day.

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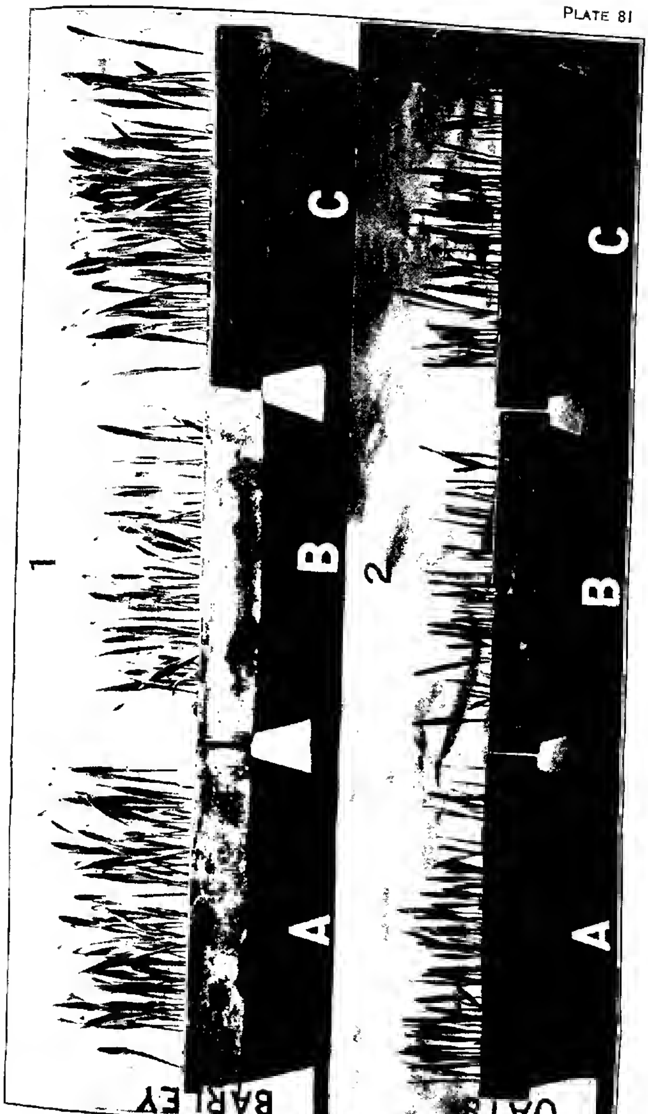
PLATE 81

Effect of presoak method on barley and oats:

1. Chevalier barley: A, control, 92 per cent germination; B, seeds treated with formalin 1 to 320 for 6 hours, 58 per cent germination; C, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 91 per cent germination.

2. Burt oats: A, control, 94 per cent germination; B, seeds treated with formalin 1 to 320 for 6 hours, 66 per cent germination; C, seeds presoaked 6 hours, then treated with formalin 1 to 320 for 6 hours, 94 per cent germination.


1
2
BARLEY
OATS
A
B
C
A
B
C

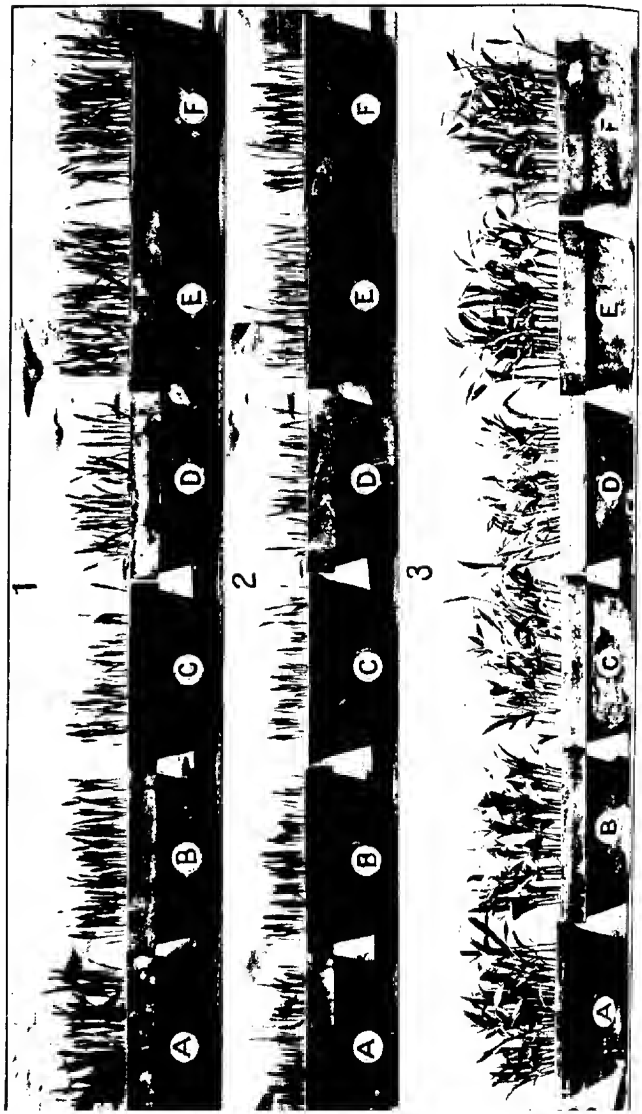

1
A
B
C
D
E
F
2
A
B
C
D
E
F
3
A
B
C
D
E
F

PLATE 82

Effect of presoak method used on barley, oats, and corn:

1. Chevalier barley: A, B, control, 92 per cent germination; C, D, seeds treated with formalin 1 to 320 for 10 minutes, then kept moist (covered) 6 hours, 62 per cent germination; E, F, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 92 per cent germination. Photographed on seventh day.

2. Burt oats: A, B, control, 96 per cent germination; C, D, seeds treated with formalin 1 to 320 for 6 hours, 71 per cent germination; E, F, seeds presoaked 6 hours, then formalin 1 to 320 for 6 hours, 92 per cent germination. Photographed on seventh day.

3. Bantam Evergreen corn: A, B, control, 94 per cent germination; C, D, seeds treated with formalin 1 to 200 for 4 hours, 90 per cent germination; E, F, seeds actually soaked in water 10 hours, then treated with formalin 1 to 200 for 4 hours, 92 per cent germination. Count taken on twelfth day, photographed on sixteenth day. The presoaked seeds are stimulated.

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